Factors Affecting the Absorption and Disposition of Frusemide and Bumetanide in man

by

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AUTHENTICATION

I declare that this thesis was composed by myself and all the work, apart from that acknowledged, is my own.

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Abstract

Drug absorption, elimination and effect may be influenced by many factors including type of formulation, particle size, dissolution and by the presence of other drugs or food and fluid ingestion. The influence of some of these factors on the absorption and effect of frusemide and bumetanide have been investigated in the present studies.

The effect of food on the absorption of oral frusemide (40 mg) was determined in 8 healthy volunteers. Food significantly reduced peak plasma concentrations (2.35 ± 0.49 versus 0.51 ± 0.19 mg/l) and delayed the time to peak. The bioavailability of frusemide was also significantly reduced from 76 % fasting to 43 % after food. The study was then repeated in 9 healthy volunteers using oral bumetanide (2 mg) given with and without food. The peak concentration was reduced after food (96.9 ± 15.1 versus 36.1 ± 11.5 µg/l) and the time to peak concentration delayed. However the mean bioavailability of bumetanide was not significantly reduced by food. A survey carried out in two medical wards within the Edinburgh Royal Infirmary then provided information on the general use of frusemide and bumetanide and showed that most of the patients were taking a single oral dose of diuretic with or in close proximity to breakfast, a situation which could potentially alter diuretic absorption and effect. Consequently, the effect of hospital breakfast on the absorption and efficacy of frusemide was studied in 10 medical inpatients. However when frusemide was administered 2 hours after breakfast, no significant improvement in area under the plasma concentration-time curve, urinary recovery of frusemide or total natriuretic and diuretic response was found.

The pharmacokinetics of frusemide were compared in 8 healthy volunteers following oral administration of single doses of 10, 40 and 80 mg. Peak plasma concentrations, areas under the plasma concentration time curves and amounts excreted, normalised to 40 mg, were not significantly different. Over this dose range there was no significant dose-dependent effect on frusemide absorption.

The effects of penicillin on frusemide renal secretion and diuretic effect were studied in 8 healthy volunteers. Penicillin significantly reduced the renal clearance of frusemide (103.0 ± 15.9 to 83.6 ± 17.4 ml/min), but had no effect on the area under the plasma concentration-time curve, total urinary recovery of frusemide or on the time course and magnitude of natriuresis and diuresis.

Finally, the time course of frusemide delivery to the active site has been suggested to be an independent determinant of overall response. The kinetic and the dynamic effects of the same total dose of frusemide (40 mg) administered as a single dose and as repeated doses over 8 h were compared in 8 healthy volunteers. The average area under the plasma concentration-time curve (2.89 ± 0.66 versus 2.25 ± 0.32 mg.h/l) and total urinary recovery of frusemide (18.3 ± 3.3 versus 16.4 ± 3.0 mg) were significantly lower following the repeated doses. However despite less drug being delivered to the active site, no significant differences were found in total sodium and water excretion compared to the single dose.

TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION AND AIMS

Gastrointestinal drug absorption may be influenced by many factors including degree of ionisation, formulation, particle size, dissolution, presence of other drugs and food or fluid ingestion (Prescott, 1969; Rowland and Tozer, 1980; Welling 1977a, b, 1984). All these factors may contribute to variability in drug response. The influence of some of these factors on the absorption , elimination and effect of frusemide and bumetanide are investigated in the following studies.

Frusemide and bumetanide are diuretic and natriuretic agents belonging to the class of loop diuretics (Ponto and Schoenwald, 1990; Ward and Heel, 1984). They are both commonly used in the treatment of oedematous states associated with cardiac, renal and hepatic disease (Hammarlund-Udenaes and Benet, 1989; Johnson and Johnson, 1990). Their pharmacokinetics have been studied extensively (Benet, 1979; Cutler and Blair, 1979; Johnson and Johnson, 1990; Ponto and Schoenwald, 1990; Ward and Heel, 1984).

Frusemide is an anthranilic acid derivative, 4-chloro-N-furfuryl-5-sulfamoylanthranilic acid, with a molecular weight of 330.74 (Cutler and Blair, 1979). The structure of frusemide is shown in Fig 1.1. The compound is unstable to light but stable in air. It is soluble in methanol, acetone, acetonitrile and solutions of alkali hydroxides. Its pKa is 3.9 (Ponto and Schoenwald, 1990).

The first clinical studies with frusemide were carried out by Kleinfelder in 1963 (Cutler and Blair, 1979) and revealed that this drug displayed the now well known characteristics of its loop diuretic activity. In contrast to thiazide diuretics, it has a shorter duration of action, steeper dose response curve and greater diuretic and

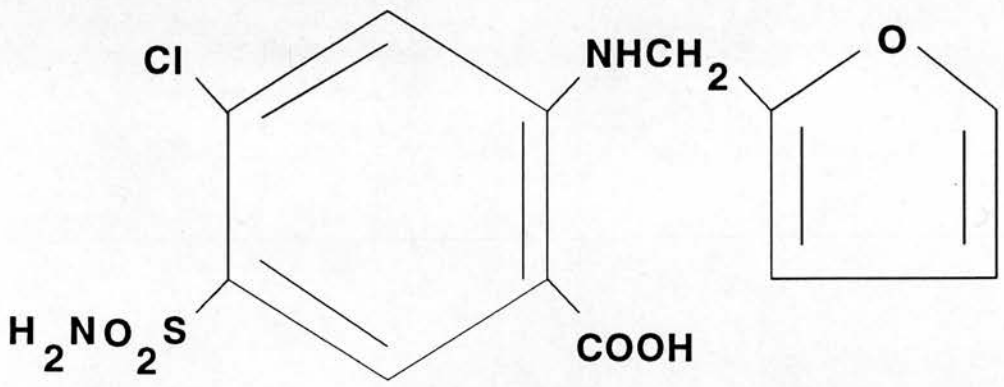


Figure 1.1. Chemical structure of frusemide

natriuretic response.

Frusemide is believed to exert its primary effect at the luminal surface of the ascending limb of the loop of Henle in the nephron (Burg, 1976; Chennavasin *et al.* 1979; Odlind, 1979; Odlind and Beermann, 1980). At this site approximately 20-30 % of the filtered sodium chloride is reabsorbed by a Na/2Cl/K cotransport system (Wittner *et al.* 1991). Frusemide is thought to reversibly block this carrier system, thus reducing sodium chloride reabsorption (Feig, 1986; Greger and Schlatter, 1983; Hendry and Ellory, 1988). The increased delivery of sodium to more distal sites of the nephron also produces an increase in the exchange of sodium for potassium, leading to greater potassium excretion. The resulting low medullary osmolality inhibits the reabsorption of water by the kidney, producing a pronounced diuresis. Although the above action is generally considered most important for the natriuretic response of frusemide (Seely and Dirks, 1977), several studies suggested that frusemide may have an additional effect at a more proximal site (Brenner *et al.* 1969; Burke *et al.* 1972; Knox *et al.* 1969; Puschett and Goldberg, 1968; Stason *et al.* 1966). This site of action was evaluated more recently using a lithium clearance technique (Beutler *et al.* 1992; Christensen *et al.* 1986, 1988). There is evidence that renal lithium clearance reflects the delivery of sodium and water out of the proximal tubule (Christensen *et al.* 1988; Thomsen, 1984). Intravenous frusemide was shown to increase lithium clearance in animals and humans suggesting that frusemide causes inhibition of sodium reabsorption in the proximal tubule (Beutler *et al.* 1992; Christensen *et al.* 1986).

In addition to its natriuretic and diuretic action, frusemide produces an early vasodilator

action in particular, a rise in peripheral venous capacitance (Dikshit *et al.* 1973; Johnston *et al.* 1983). This extrarenal vascular effect occurs prior to any diuretic effect and is dependent on sodium balance and renal function (Johnston *et al.* 1983). Inhibition of prostaglandin production also appears to abolish any increase in venous capacitance produced by frusemide (Bourland *et al.* 1977; Johnston *et al.* 1983). Frusemide also reduces renal vascular resistance with a resultant increase in renal blood flow, the degree of which is proportional to the initial resistance (Ludens *et al.* 1968). During diuresis, glomerular filtration rate increases (Passmore *et al.* 1989), decreases (Christensen *et al.* 1986) or remains unaltered (Dupont *et al.* 1988).

Plasma concentrations of angiotensin II, aldosterone and noradrenaline increase after frusemide administration (Mackay *et al.* 1984; Sjöstrom *et al.* 1988a,b; Wilcox *et al.* 1983). After intravenous administration, the increase is thought to be independent of sodium loss and the reduction in plasma volume whereas the release after oral administration seems to be related to cumulative sodium excretion (Francis *et al.* 1985; Noda *et al.* 1982). The acute natriuresis of frusemide is succeeded by a period of sodium retention, a process referred to as acute tolerance (Andreasen *et al.* 1989; Hammarlund *et al.* 1985; Wilcox *et al.* 1983). Since angiotensin II (Johnson and Malvin, 1977) and aldosterone can increase renal sodium reabsorption (Kelly *et al.* 1983) it has been suggested that the observed activation of the renin-angiotensin-aldosterone system may contribute to the development of acute tolerance (Sjöstrom *et al.* 1988b).

In humans and animals, excretion of urinary prostaglandins also increase after frusemide administration (Abe *et al.* 1977; Dupont *et al.* 1988; Katayama *et al.* 1984; Passmore *et*

al. 1989; Patak *et al.* 1979) and in the rat frusemide has been shown to directly stimulate prostaglandin E_2 production in the thick ascending limb of the loop of Henle (Miyanoshita *et al.* 1989). Since prostaglandins A and E exhibit natriuretic and diuretic effects, they may play a role in the response to frusemide. Prostaglandin synthetase inhibitors such as indomethacin have been shown to attenuate both the haemodynamic and diuretic effects of frusemide in most experimental studies (Benet, 1979; Chennavasin *et al.* 1980; Errikson *et al.* 1987; Greven and Farjam, 1988; Nies *et al.* 1983; Patak *et al.* 1975) but not all (Bailie *et al.* 1976; Data *et al.* 1978; Williamson *et al.* 1975).

Being a weak acid, frusemide is believed to reach its site of action in the loop of Henle by active secretion in the proximal tubule via the nonspecific organic acid pump (Calesnick *et al.* 1966; Forrey *et al.* 1974; Odlind, 1979). This is supported by the fact that blockage of the pathway by probenecid, a competitive inhibitor of the organic acid pump (Cunningham *et al.* 1981), decreases the renal clearance and urinary excretion of frusemide (Brater, 1978; Chennavasin *et al.* 1979; Homeida *et al.* 1977; Honari *et al.* 1977; Odlind and Beermann, 1980). This change causes a rightward shift in the relationship between serum concentrations of frusemide and response while having no effect on the relationship between urinary frusemide and response (Chennavasin *et al.* 1979), demonstrating that the response is related to the concentration of drug in the urine as opposed to that in the plasma in normal man.

The oral absorption of frusemide in healthy volunteers is known to be erratic and is subject to large inter- and intraindividual variation (Andreasen *et al.* 1982; Grahnen *et*

al. 1984). The bioavailability from oral dosage forms is also highly variable with reported values ranging from 20 - 84 % (Grahnen *et al.* 1984). The extent of absorption is influenced by underlying disease processes, being decreased in renal disease and even more erratic in heart failure (Brater *et al.* 1982a; Greither et al. 1979; Ponto and Schoenwald, 1990). Absorption is also variably influenced by dosage form (Hammarlund *et al.* 1984; Waller *et al.* 1988) and food (Beermann and Midskov, 1986; Hammarlund *et al.* 1984; Kelly *et al.* 1973). Hammarlund *et al* (1984) and Waller *et al* (1988) found differences in the rate of frusemide absorption between a tablet and solution form, however the extent of absorption was similar. Kelly *et al* (1973) and Hammarlund *et al* (1984) found no difference in the extent of absorption when frusemide was administered with food. Hammarlund *et al* (1984) did find that food delayed its absorption by approximately 60 minutes. On the other hand Beermann and Midskov (1986) found that food reduced the bioavailability of frusemide by approximately 30 %.

It was suggested that the poor bioavailability could be due to poor solubility of frusemide, gastric first pass metabolism (Lee *et al.* 1983) and possibly frusemide site specific active absorption (Chungi *et al.* 1979; Ritschel *et al.* 1991).

One, two and three compartment models have been used to describe the plasma concentration-time curves following intravenous administration of frusemide (Cutler and Blair, 1979; Hammarlund *et al.* 1984; Kelly *et al.* 1973). However a 2-compartment model is most often used due to the relatively minor contribution of the third process to the overall area under the plasma concentration time curve (Hammarlund-Udenaes and

Benet, 1989). Using this model the early distribution phase is quite rapid following an intravenous dose and usually appears to be complete within 30 minutes (Cutler and Blair, 1979)

Frusemide is extensively bound to plasma proteins (> 90 %), almost exclusively to albumin (Andreasen and Jakobsen, 1974; Bowman, 1975; Cutler *et al.* 1974; Rane *et al.* 1978). The high degree of protein binding restricts the apparent volume of distribution of the drug and values of 4.6 - 13.8 litres have been reported in healthy volunteers (Ponto and Schoenwald, 1990).

A glucuronide metabolite of frusemide is excreted in the urine and faeces (Ponto and Schoenwald, 1990) however the fraction excreted in the urine varies, ranging from 2 - 25 % in healthy volunteers (Andreasen and Mikkelson, 1977; Andreasen *et al.* 1981; Rakhit *et al.* 1987; Smith *et al.* 1980). Factors such as age, route of administration and long term use may contribute to the variability (Andreasen and Mikkelson, 1977; Beermann *et al.* 1975; Hammarlund-Udenaes and Benet, 1989). After intravenous administration, between 6 and 18 % of the dose will eventually be eliminated in the faeces of healthy subjects (Beermann *et al.* 1975). The site or sites of frusemide metabolism are still unknown, although it has been suggested that the liver is probably not the major site since there is no change in nonrenal clearance in patients with liver disease (Fuller *et al.* 1981; Verbeeck *et al.* 1982). Also a study in dogs demonstrated that neither the renal or nonrenal clearance of frusemide was changed when the entire liver was removed and that urinary recovery of frusemide glucuronide remained unchanged (Verbeeck *et al.* 1981). The kidney itself has been proposed as the site of

glucuronidation (Rakhit *et al.* 1987; Vree *et al.* 1995).

There is much debate over another potential metabolite, 4-chloro-5-sulfamoyl anthranilic acid (CSA). Several investigators have argued that CSA is an analytical artifact produced during extraction procedures (Benet, 1979; Smith *et al.* 1980). This was due to the fact that some investigators failed to find CSA in the plasma and urine of healthy volunteers and patients (Beermann *et al.* 1975; Branch *et al.* 1977; Kerremans *et al.* 1982; Smith *et al.* 1980) and that a major photodegradation product of frusemide glucuronide had a similar retention time as CSA under certain analytical conditions (Hammarlund-Udenaes and Benet, 1989). Smith *et al* (1980) were also able to produce CSA from acid degradation of a frusemide stock solution as well as by employing the acid extraction procedure detailed by Perez *et al.* (1979).

In healthy volunteers the elimination half-life of frusemide is usually in the range 30 - 120 minutes (Ponto and Schoenwald, 1990). Huang *et al* (1974) found an average half-life of 9.7 h in patients with end stage renal disease. When compared to healthy volunteers, the elimination half-life was longer or equivalent in patients with liver disease (Dreux *et al.* 1979; Verbeeck *et al.* 1982) and the range reported in patients with congestive heart failure is 50 - 330 minutes (Brater *et al.* 1982a).

In healthy and diseased subjects the systemic clearance of frusemide is generally reported to be in the range 0.09 - 0.18 L/kg and renal and nonrenal processes generally contribute equally (Benet, 1979). The relative contribution of the excretion processes is influenced by age, disease and drug interactions (Ponto and Schoenwald, 1990). Clearance reduces with age, probably due to declining renal function (Chaudry *et al.*

1984; Kerremans *et al.* 1983). Impaired renal function in patients with renal and cardiac failure decreases the renal component of the systemic clearance of frusemide (Brater *et al.* 1982a; Rane *et al.* 1978; Rose *et al.* 1976). Probenecid and indomethacin reduce the renal clearance of frusemide by inhibiting its active secretion (Odlind and Beermann, 1980; Homeida *et al.* 1977; Honari *et al.* 1977; Smith *et al.* 1979, 1980).

The effectiveness of frusemide as a diuretic depends upon it reaching its site of action, the renal tubules, unchanged (Brater, 1986). Approximately one-half to two-thirds of an intravenous dose or one-quarter to one-half of an oral dose are excreted unchanged, the difference being largely due to poor bioavailability from the oral route (Ponto and Schoenwald, 1990).

Bumetanide (3-n-butylamino-4-phenoxy-5-sulfamylbenzoic acid) was first synthesised in 1968 (Johnson and Johnson, 1990). Its structure is shown in Fig 1.2. Olesen *et al* (1973) carried out the first clinical study which proved the usefulness of this drug in patients with congestive heart failure and in the same year Feit *et al* (1973) developed a sensitive gas liquid chromatography technique for its measurement. In healthy subjects bumetanide is 40 - 70 times more potent than frusemide on a weight basis (Brater *et al.* 1981, 1983a; Davies *et al.* 1974; Ramsay *et al.* 1978).

Like frusemide, bumetanide is believed to exert its primary affect at the thick ascending limb of the loop of Henle (Bourke *et al.* 1973; Higashio *et al.* 1978; Lant , 1975). It binds reversibly to the Na/2Cl/K transporter complex at a protein binding site for chloride (Hass and Forbush, 1987; Hass and McManus, 1983; Hedge and Palfrey, 1992). By this reversible binding it inhibits sodium and chloride reabsorption. Bumetanide also

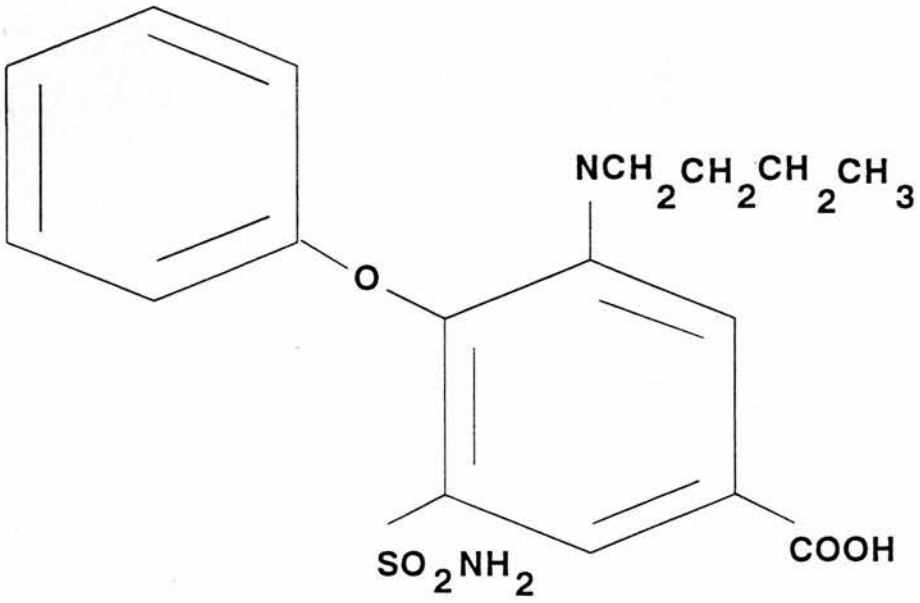


Figure 1.2. Chemical structure of bumetanide

reduces sodium reabsorption in the proximal tubule (Puschett *et al.* 1978; Staalsen and Steiness, 1990), an action which seems to be independent of carbonic anhydrase inhibition (Jayakumar and Puschett, 1977; Vogh and Langham, 1981). Bumetanide causes less potassium loss than frusemide, for a given natriuresis, in healthy volunteers (Branch *et al.* 1976; Hettiarachchi *et al.* 1977; Ramsay *et al.* 1978).

Bumetanide enhances renal blood flow but has little effect on glomerular filtration rate in man (Bourke *et al.* 1973; Olsen, 1975a). The renal blood flow effect occurs before diuresis and is inhibited by indomethacin. It is therefore thought to involve prostaglandins (Olsen, 1975 b). The observation that the pattern of urinary excretion of prostaglandin E is similar to that of enhancement of renal blood flow also suggests this (Ward and Heel, 1984). Bumetanide, like other loop diuretics, also causes a several fold increase in plasma renin activity in animals (Olsen and Ahnfelt-Ronne, 1976) and man (Pedrinelli *et al.* 1980).

Absorption of bumetanide is rapid and almost complete following a single oral dose in healthy volunteers (Marcantonio *et al.* 1982; Pentikainen *et al.* 1977). Using ^{14}C-labelling or high performance liquid chromatography techniques, bioavailabilities of 90 % or more have been recorded (Halladay *et al.* 1977; Pentikainen *et al.* 1977). However lower values of approximately 80 % have also been reported (Brater *et al.* 1983a; Cook *et al.* 1988; Holazo *et al.* 1984). Bumetanide absorption also seems to be unaffected by disease states (Bailie *et al.* 1987; Cook *et al.* 1988; Marcantonio *et al.* 1983) although slower absorption has been reported in patients with congestive heart failure (Brater *et al.* 1982b).

The oral bioavailability of bumetanide is therefore about 80 % compared with 50 % for frusemide. This distinction is important when converting from intravenous to oral administration. Changing from intravenous to oral dosing requires twice as much oral frusemide, whereas equal doses are required for bumetanide (Brater, 1986). The time course of absorption does not differ appreciably between the two diuretics (Brater *et al.* 1983a).

The plasma concentration time curves for bumetanide are most often described using a 2 compartment model (Bailie *et al.* 1987; Pentikainen *et al.* 1980) however monoexponential and triexponential models have also been used (Davies *et al.* 1974; Pentikainen *et al.* 1980). After intravenous administration in healthy subjects, bumetanide has a rapid distribution phase followed by slower elimination with a half-life of 1 - 1.5 h (Marcantonio *et al.* 1982; Pentikainen *et al.* 1977, 1980). The values reported for the apparent volume of distribution of bumetanide in healthy volunteers range from 9.5 to 35 litres (Davies *et al.* 1974; Dixon *et al.* 1976; Marcantonio *et al.* 1982). Its high degree of protein binding (> 90 %) is responsible for limiting the volume of distribution (Walker *et al.* 1989).

In healthy volunteers, bumetanide is cleared from the plasma at an average rate of 200 to 250 ml/min. Renal elimination constitutes about half of the clearance (Davies *et al.* 1974; Dixon *et al.* 1976; Halladay *et al*, 1977; Pentikainen *et al,* 1980) and hepatic metabolism and biliary excretion account for the other half. Filtration of bumetanide is limited by the high degree of protein binding, however being a weak acid it is readily secreted via the non specific organic acid pathway of the proximal tubule (Odlind *et al.*

1983). Approximately 60-70 % of an intravenous dose is eliminated by this route and appears unchanged in the urine (Cook *et al.* 1988; Lau *et al.* 1986: Marcantonio *et al.* 1982). The remainder is metabolised via oxidation of the n-butyl side chain to several metabolites (Halladay *et al.* 1975; Pantikainen *et al.* 1977) which are devoid of diuretic activity (Schwartz, 1981). Most of the metabolites are subsequently excreted in the urine, however a small amount is eliminated in the faeces via biliary excretion (Halladay *et al.* 1975; Pentikainen *et al.* 1977, 1985). Differences therefore also exist in the metabolism of frusemide and bumetanide. The site of bumetanide metabolism is hepatic whereas that of frusemide may be renal in origin (Beermann *et al.* 1975; Verbeeck *et al.* 1981). Also, bumetanide has several metabolites (Halladay *et al.* 1975), while frusemide may only have a single glucuronide metabolite (Beermann *et al.* 1975; Smith *et al.* 1980).

From a pharmacokinetic perspective, the overall responses to frusemide and bumetanide depend upon the total amount of drug delivered to the site of action, which will in turn be determined by the dose, quantitative absorption and the capacity of the organic acid transport pump to deliver drug from the blood into the urine (Brater, 1985). Any factor which has the potential to decrease or increase absorption or excretion could therefore alter diuretic response accordingly.

Food is one factor which exerts complex and often unpredictable influences on the oral bioavailability of drugs (Beermann, 1979; Melander, 1978; Toothaker and Welling, 1980; Welling, 1977a, b, 1984). The outcome of drug food interactions is likely to be the overall effect resulting from a number of factors related to the influence of food on

gastrointestinal function or to a direct interaction between food components and the drug (Welling, 1984).

The predominant effect of food is inhibition of stomach emptying due to feedback mechanisms from receptors situated in the small intestine (Melander, 1978). Since most drugs are predominantly absorbed from the small intestine (Rowland and Tozer, 1980), changes in stomach emptying rate are likely to affect the rate of drug absorption. Prolonged residence time in the acidic environment of the stomach may however have other effects on drug absorption. It may delay the dissolution of acidic drugs and accelerate dissolution of basic compounds (Welling, 1977 b). Ingestion of food increases gastric secretion of hydrochloric acid, digestive enzymes and bile, hence increased residence time may cause a reduction in the absorption efficiency for drugs which are either acid labile or sensitive to such enzymes (Beermann, 1979). On the other hand drugs absorbed by saturable mechanisms or from specific intestinal sites may exhibit increased absorption due to a decrease in the rate at which the drug passes its absorption site (Toothaker and Welling, 1980). In addition to changes in absorption resulting from physiological effects, altered absorption may also result from direct interactions between food components and drugs, generally resulting in decreased bioavailability. Food may also act as a purely physical barrier preventing a drug from reaching the gastrointestinal surface (Beermann, 1979).

As judged mainly from single meal, single dose studies in healthy volunteers, food has been shown to increase the bioavailability of several antihypertensive drugs. Areas under the plasma concentration time curves for propranolol, metoprolol and hydralazine

were significantly larger when administered with food compared to the fasting state (Melander *et al.* 1977a, b). There is evidence that the gastrointestinal absorption of propranolol and metoprolol is virtually complete under fasting conditions (Shand, 1974), and it was therefore suggested that food intake somehow reduced their first pass metabolism in the liver (Melander, 1977a, Melander and McLean, 1983). A similar mechanism may explain the increased bioavailability of hydralazine (Melander, 1978). The amount of hydrochlorothiazide recovered in the urine was also increased when given with food (Beermann and Groschinsky-Grind, 1978). A possible explanation was that food, by inhibiting stomach emptying, decreased tha rate at which the drug passed its absorption site in the upper small intestine. Other drugs incuding diphenylhydantoin (Melander *et al.* 1979a) and nitrofurantoin (Rosenberg and Bates, 1976) also show increased absorption following administration with food. All orally administered drugs must be in solution before they can be absorbed (Rowland and Tozer, 1980) and a possible explanation put forward for both drugs was that delayed stomach emptying allowed for more effective tablet disintegration and drug dissolution in the stomach before they pass into the optimal absorption site of the small intestine (Bates, 1974; Melander *et al.* 1979a). Griseofulvin absorption is increased by high fat meals but not by high protein or carbohydrate meals in normal individuals (Welling, 1977b). This is most probably explained by fat induced enhancement of the dissolution of griseofulvin, which is extremely lipophilic (Bates *et al.* 1966).

In contrast, several antimicrobial agents have reduced bioavailability when administered with food. The absorption of oxytetracycline and tetracycline is drastically reduced by the intake of calcium rich foods such as milk and cheese due to chelates forming between

the metals and the tetracyclines (Neuvonen and Turakka, 1974; Neuvonen 1976). Food has also been reported to reduce the absorption of penicillinV, penicillin G, penicillamine, ampicillin and oxacillin (McCarthy and Finland. 1960; Osman *et al.* 1982; Welling, 1977b). On the other hand the bioavailability of amoxycillin seems unaffected by food (Melander, 1978). Peak plasma concentrations and areas under the plasma concentration-time curve of isoniazid were also significantly decreased by food (Melander *et al.* 1976) and delay in gastric emptying consequent to food was proposed as the explanation although direct interaction of isoniazid and food components may also be important (Melander *et al.* 1976). Other drugs whose bioavailability is reduced by food include atenolol (Melander *et al*, 1979b), captopril (Singhvi *et al.* 1982) and ketoconazole (Mannisto *et al.* 1982).

For drugs such as diazepam, digoxin and quinidine, the rate of drug absorption is delayed but the total quantity of drug available to the systemic circulation is not reduced when either drug is ingested with a meal (Greenblatt *et al.* 1974; Greenblatt *et al.* 1978; Johnson *et al.* 1978; Woo and Greenblatt, 1980). Wessels *et al* (1992) showed that breakfast with a high fat content delayed the absorption of paracetamol. Breakfast with a high carbohydrate or cereal content influenced the rate of absorption to a lesser extent. Food intake seemed to reduce the rate of paracetamol absorption due to retarded gastric emptying (Heading *et al.* 1973; McGilveray and Mattok, 1972; Nimmo *et al.* 1973, 1975).

Concomitant food intake appears to have no apparent influence on the absorption and bioavailability of drugs such as oxprenolol (Dawes *et al.* 1979), bendroflumethaizide

(Beermann *et al.* 1978) and oxazepam (Melander *et al.* 1977 c).

The formulation in which a drug is administered also has a profound effect on the extent of a particular drug-food interaction. While the uptake of nitrofurantoin from tablets is enhanced when administered with food, the absorption of the drug from a suspension is unaffected (Rosenberg and Bates, 1976). The absorption of aspirin from conventional tablets appears to be reduced by food (Spiers and Malone, 1967) but absorption from effervescent tablets is only slight delayed (Volans, 1974). Finally, the uptake of two esters of erythromycin is also decreased when given as a tablet with food (Hirsch and Finland, 1959) however it remains unaffected when administered as a suspension (Hirsch *et al.* 1960).

Food ingestion can therefore increase, decrease, delay or have no effect on drug absorption. Also different food components may have different effects, different preparations of the same drug may interact differently with food and food may interact in opposite ways, even with drugs that are chemically related. Therefore, the net result of food on drug bioavailability can only be derived from direct studies of the drug in question.

Although studies in healthy subjects have previously been carried out to determine the effects of food on oral frusemide absorption, the results have been variable. Some suggested that its absorption may only be delayed by the intake of breakfast (Kelly *et al.* 1973; Hammarlund *et al.* 1984), while another found that breakfast significantly reduced its bioavailability to an extent where efficacy may be impaired (Beermann and Midskov, 1986). No definitive studies of the effects food on bumetanide absorption have been

reported. In light of this information, the unpredictable nature of food on drug bioavailability and the fact that many patients take medication at mealtimes, the effect of food on frusemide and bumetanide absorption in man has been compared.

Because patients receiving medication may also receive more than one drug at a time, interactions between drugs represents another major problem in drug therapy. Since the sites of frusemide action are primarily reached via the organic acid secretory pump at the proximal tubule (Andreasen *et al.* 1978; Calesnick *et al.* 1966; Odlind, 1979), the presence of other coadministered acids may interfere with its transport into the tubular lumen. It was therefore decided, in another study, to detemine the effects of intravenous penicillin , another commonly used drug also secreted via the proximal tubules, on the renal elimination and response of frusemide. This potential drug-drug interaction has not previously been investigated. As mentioned earlier, numerous studies in animals and man (Brater, 1978; Chennavasin *et al.* 1979; Friedman and Roch-Ramel, 1977; Homeida *et al.* 1977; Honari *et al.* 1977; Hook and Williamson, 1965; Odlind,1979; Sommers *et al.* 1991) have previously shown that probenecid pretreatment significantly reduces the renal clearance of frusemide (up to 78 % in the study by Homeida *et al.* 1977) by competing for renal secretion.

An unexpected finding from some of these studies (Brater, 1978; Chennavasin *et al.* 1979; Sommers *et al.* 1991) however was that pretreatment of healthy subjects with probenecid increased the overall natriuretic response to frusemide. Since the dose response curves were found to be identical, the effect did not occur because of changed sensitivity of the nephron to frusemide (Brater, 1978).

A similar phenomenon has been found in studies comparing overall response to oral and intravenous frusemide (Branch, 1977; Kaojarern *et al.* 1982; Kelly *et al.* 1973). Branch *et al* (1977) found that the natriuretic response by either route was virtually the same, despite approximately half as much frusemide reaching the site of action with the oral dose compared to the intravenous dose. Kelly *et al* (1973) also compared the response between different formulations and routes of administration of frusemide in healthy volunteers. They again found no significant differences in total sodium excretion after oral versus intravenous administration, while the oral bioavailability of drug was approximately half that after intravenous administration. As with the probenecid effect there was a discrepancy between overall response relative to the amount of drug reaching the active site.

Kaojarern *et al* (1982) suggested that in addition to frusemide absorption and the total amount of drug in the urine, the time course of delivery of frusemide into the urine was another independent determinant of overall response. They calculated a maximally efficient excretion rate for frusemide and found that amounts of frusemide after an oral dose more persistently approached the amount with maximal efficiency compared to an intravenous dose. In accordance with this concept several investigations in healthy volunteers found that intravenous infusion of frusemide led to a better response than bolus injection (Lahav *et al.* 1992; Meyel *et al.* 1992).

If small repeated oral doses of frusemide were given at regular intervals, steady levels of the drug may be obtained which may also result in greater cumulative sodium excretion. Consequently, a slow release oral preparation of frusemide may be a more efficient way

to administer such a drug. This hypothesis was tested by comparing the pharmacokinetic and dynamic effects of a single oral dose of frusemide with an equivalent dose administered at hourly intervals in healthy subjects.

The information obtained from the following studies may lead to the more effective use of frusemide and bumetanide in clinical practice, in particular, by controlling the times of administration in relation to meals.

Aims

The following studies were undertaken to:

1) Determine the effects of food on frusemide absorption in healthy volunteers

2) Determine the effect of food on bumetanide absorption in healthy volunteers

3) Obtain information on the general use of frusemide and bumetanide in two medical wards within the Edinburgh Royal Infirmary

4) Establish the effect of hospital breakfast on the absorption and efficacy of frusemide in hospital patients

5) Determine whether frusemide absorption was dose-dependent

6) Study the effects of penicillin on frusemide renal secretion and diuretic effect

7) Compare the pharmacokinetic and pharmacodynamic effects of the same total dose of frusemide administered as a single dose and as repeated small doses in healthy volunteers

CHAPTER 2

METHODS

Section 2.1: Analytical Methods

Measurement of frusemide in plasma and urine by high performance liquid chromatography

Background

Over the years many methods have been developed for the analysis of frusemide in biological samples. The earliest were based on spectrophotometry (Haussler and Hadjú, 1964), others employed gas chromatography (Lindstrom and Molander, 1974) and thin layer chromatography (Steiness *et al.* 1979; Yakatan *et al.* 1976). Subsequently, in order to improve sensitivity, several high performance liquid chromatography methods were developed (Andreasen *et al.* 1981; Carr *et al.* 1978; Kerremans *et al.* 1982; Lin *et al.* 1979; Nation *et al.* 1979; Rapaka *et al.* 1982; Sood *et al.* 1987; Uchino *et al.* 1984). The reason for such extensive literature regarding the analysis of frusemide relates to problems associated with its stability.

Several methods used liquid-liquid extraction before chromatography (Andreasen *et al.* 1981; Carr *et al.* 1978; Lindstrom, 1974; Perez *et al.* 1979; Steiness *et al.* 1979). Typically, samples were acidified with hydrochloric acid before extraction with ether. The organic phase was then evaporated and the residue dissolved in a suitable solvent for injection. Acidification of the samples was necessary to separate frusemide from plasma protein and transfer it to the organic layer. However, under acidic conditions degradation of frusemide occurs with the formation of 4-chloro-5-sulphamoylanthranilic acid (CSA)(Cruz *et al.* 1979; Kovar *et al.* 1974; Rowbotham *et*

al. 1976), complete transformation being achieved by heating an acid solution of frusemide to 70^0 for 45 minutes (Fig 2.1) (Hadju & Haussler, 1964). Using 1.5M hydrochloric acid as acidifying agent Kerreman *et al* (1982) obtained a recovery of frusemide of 50 % because of degradation. However no degradation of frusemide was observed using acetic acid as the acidifying agent. Also Smith *et al* (1980) using a urine sample known to contain frusemide but no CSA were able to detect a CSA peak after applying the acid extraction procedure described by Perez *et al* (1979). Using their own direct injection method, no CSA was detected in any of the samples analysed. This degradation of frusemide may be even faster if sulphate ions are present as in human urine (Shah *et al.* 1980).

This problem is further complicated by the fact that the fluorescence of frusemide is optimal at low pH. Using frusemide made up in phosphate buffers of varying pH, Blair *et al* (1975) discovered that the relative fluorescent intensities for frusemide were 100 % at pH 1.6, 97 % at pH 2.5 and 81 % at pH 4.6. At pH 5.6 and 6.6 frusemide did not fluoresce. However, despite the necessity to bring samples into an acid pH range, fluorescence detection offers much greater sensitivity and has been favoured by several investigators (Kerremans *et al.* 1982; Lovett *et al.* 1985; Rapaka *et al.* 1982; Smith *et al.* 1980).

The stability of frusemide to light has also been questioned (Moore and Sithipitaks, 1983). Several methods have recommended exclusion of light from biological samples (Sood *et al.* 1987; Steiness *et al.* 1979; Uchino *et al.* 1984), mostly without any further explanation. However Kerreman *et al* (1982) demonstrated that fast degradation took

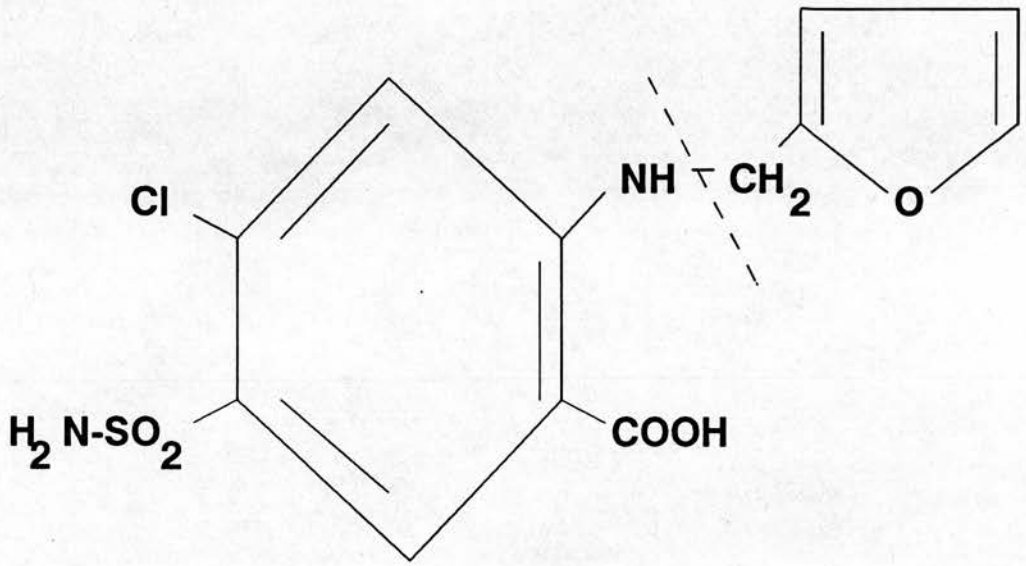


Figure 2.1. Frusemide (4 chloro-N-furfuryl-5-sulfamoylanthranilic acid). The dashed line indicates the degradation resulting from exposure to heat and acid. The split product CSA is left of the dotted line

place when solutions of frusemide in phosphate buffers of varying pH were exposed to light. Similar results were obtained with frusemide in human urine. When the same samples were protected from light 100 % recovery of frusemide was obtained. On the other hand Lovett *et al* (1985) found it unnecessary to protect their samples from light during sample extraction. Frusemide stability was tested in each of the solvents used in the assay and no photolytic degradation was observed.

The studies above outline the degradation problems in handling frusemide but also suggest that they may be prevented by taking appropriate precautions. The analytical method described below avoids the use of strong acids and light has been excluded from samples where possible.

Method

Frusemide concentrations in plasma and urine were measured by High Performance Liquid Chromatography (HPLC) with fluorescence detection. The components of the HPLC system used are shown in Fig. 2.2.

Measurement of frusemide in plasma

Frusemide was extracted from plasma using a solid phase extraction technique developed by Russel *et al.* (1989). The extraction is based on the selective adsorption of frusemide onto covalently bonded silica (C_{18} "Bond Elut") columns (see Fig 2.3). Samples are drawn through the columns using a vacuum system ("Vac Elut SPS 24"). Components of the Vac Elut system are given in Fig. 2.4.

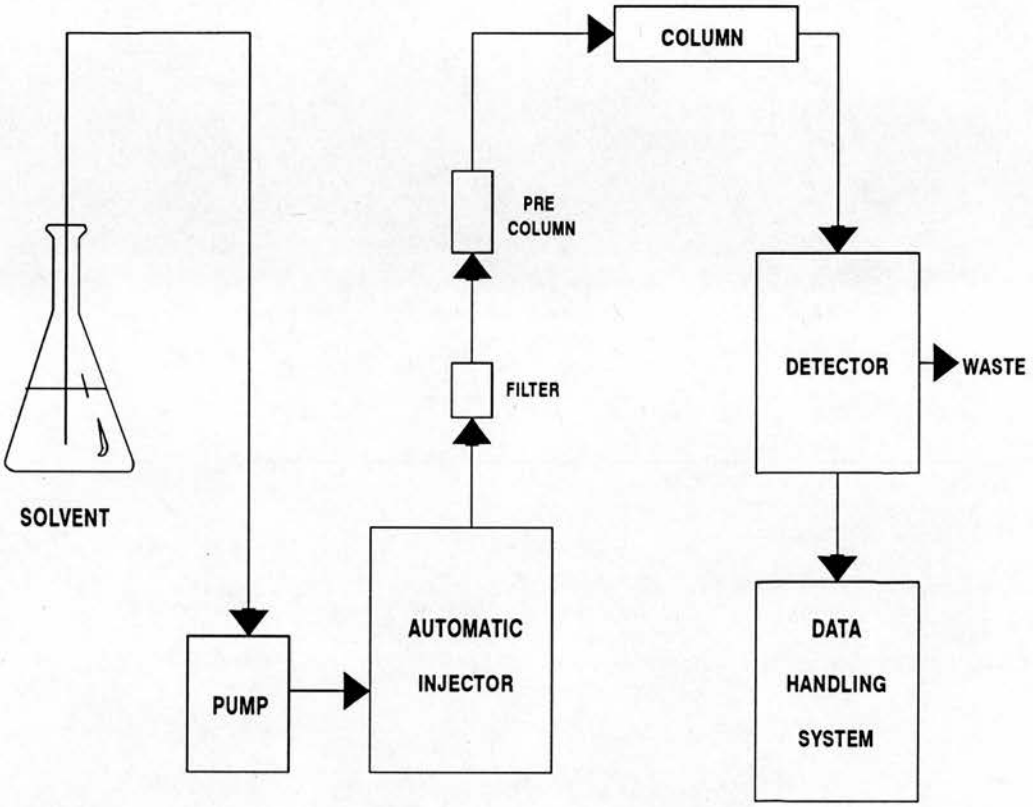


Figure 2.2. High performance liquid chromatography system used for the measurement of frusemide in plasma and urine

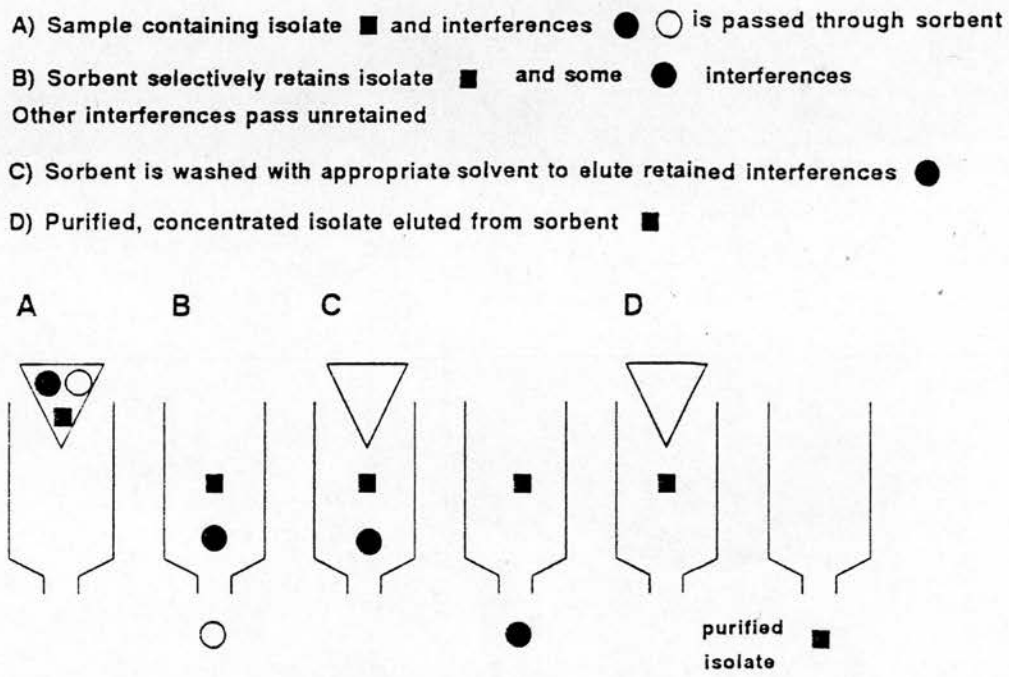


Figure 2.3. Diagram explaining the stages of solid phase extraction

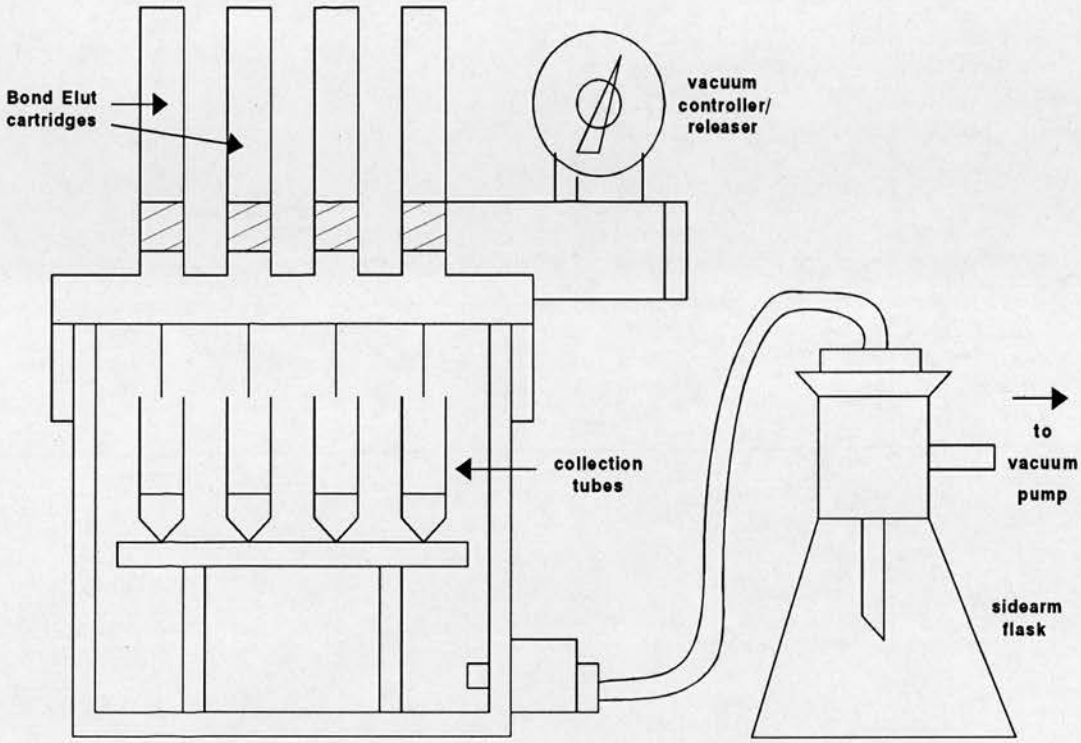


Figure 2.4. Components of the "Vac Elut" system used during solid phase extraction. The system accepts up to 24 Bond Elut columns.

Materials

Desmethylnaproxen, the internal standard, was kindly supplied by Syntex (Riccarton, Edinburgh). Potassium dihydrogen orthophosphate, citric acid, orthophosphoric acid, potassium hydroxide and urea were all supplied by BDH Chemicals Limited (Poole, England). Methanol was HPLC grade. "Bond Elut" columns were manufactured by Varian (Harbor City, USA) and supplied by Jones Chromatography (Mid-Glamorgan, Wales).

Plasma Standards

Stock solutions (1 g/l) of frusemide and desmethylnaproxen (DMN) in methanol were prepared in amber standard flasks. Standard solutions of frusemide were prepared by dilution of the stock using drug free plasma. Two standard ranges were used, 0.05 - 0.5 mg/l and 0.05 - 2 mg/l. A blank plasma standard was included in each range. 100 µl of 2 mg/l and 100 µl of 10 mg/l DMN were used as the internal standards. All standards were protected from light by wrapping aluminium foil around the containers and were stored in a freezer at approximately -20^{0}C.

Sample Pretreatment

Prior to analysis all standards and unknowns were pretreated as follows. 100 µl of DMN were pipetted into 10 ml amber test tubes. The DMN was evaporated to dryness under a stream of nitrogen at room temperature. Aliquots of 0.5 ml of plasma and 1.5 ml of a 50% (w/w) aqueous solution of urea were successively pipetted into each tube. The tubes were vortex mixed for 1 second and allowed to stand for 10 minutes. Urea

denatures the protein in plasma to which frusemide is extensively bound (>95%) (Andreasen *et al.* 1974; Forrey *et al.* 1974; Smith *et al.* 1980). 2 ml of a 0.01 M potassium citrate buffer (pH 3.0) were then added to the tubes. Finally the tubes were vortex mixed for 1 s. The samples were then ready to be loaded onto the "Bond Elut" solid phase extraction columns.

Non polar Sorbent Extraction

Compounds with non polar functional groups can be extracted from polar solutions using a non polar sorbent. The sorbent used was non-polar C_{18} octadecyl silica (ODS). Prior to use the "Bond Elut" columns were solvated which creates an environment suitable for isolate retention. This was accomplished by drawing 3 ml of methanol followed by approximately 3 ml of 0.01 M potassium citrate buffer (pH 5.0) through each column. The prepared plasma samples were then loaded onto the columns and drawn through. The columns were washed with 10 ml of the same pH 5.0 buffer. Frusemide and DMN were eluted from the sorbent using 1 ml of a 50:50 mixture consisting of 0.01 M aqueous sodium hydrogen carbonate and methanol (pH 9.2). The eluates collected were ready for HPLC analysis.

Apparatus and Chromatographic Conditions

The HPLC system consisted of a Waters 510 HPLC pump which delivered the mobile phase to an automatic sample injection module, the Waters Intelligent Sample Processor (710B). Separation was carried out using a Radial Compression reverse phase column (Nova Pak C_{18} ODS, 4µm). The column was kept at a constant temperature of 40^0C

using a Waters chromatography oven and was protected with both a metal filter and a precolumn packed with Nova-Pak C_{18}. Both frusemide and DMN were measured with a Perkin Elmer Luminescence Spectrophotometer. The excitation wavelength was set at 275 nm and the emission wavelength at 400 nm. The signal from the spectrophotometer was detected by a DCS computer running a JCL6000 Chromatography Data System which provided full analysis of the chromatograms. The mobile phase consisted of methanol and 0.02 M phosphate buffer pH 3.0 (48:52). It was degassed with helium before use and was delivered at a constant rate of 1 ml/min.

Relative Recovery

The recovery of frusemide was determined as follows. 0.5 and 2 mg/l frusemide solutions were prepared in methanol. 0.5 ml of each solution was mixed with 100 µl of 10 mg/l DMN and evaporated to dryness under a stream of nitrogen at room temperature. The residues were then reconstituted in 1 ml of the 50:50 mixture of methanol and sodium hydrogen carbonate and injected into the HPLC system. Plasma samples also containing 0.5 and 2 mg/l frusemide were extracted as previously described and then injected onto the column. Recovery with the extraction procedure was determined by comparing the peak height ratio (frusemide/DMN) obtained after injection of extracted plasma with the peak height ratio obtained after injection of non-extracted solutions of frusemide in methanol.

Stability Study

Stability studies were performed on plasma samples containing 0.5 and 2 mg/l

frusemide. The samples were either left on the bench, refrigerated at 4^0C or frozen at -20°C. Peak height ratios were compared with an original value obtained on the day the standards were prepared.

Results

Chromatograms

The total run time for each sample was 6 minutes in which frusemide and DMN were eluted from the column with retention times of approximately 3.3 and 4.4 minutes respectively. Fig 2.5 shows typical chromatograms obtained for drug free plasma and for plasma containing 0.2 and 1.2 mg/l frusemide.

Standard Curves

For each standard a peak area ratio was calculated by dividing the area of the frusemide peak by the area of the DMN peak. Plots were drawn of peak area ratio versus frusemide plasma concentration. The relationship was linear over the frusemide concentration range 0.05-1 mg/l (r= 0.9991, n=5) and 1-10 mg/l (r=0.9998, n=5). The 0.05 - 0.5 and 0.05 - 2 mg/l ranges of plasma standards were assayed on five separate days. The results are shown in Tables 2.1 and 2.2 and Figs 2.6 and 2.7. The coefficient of variation of the assay, calculated from five replicate determinations was less than 10 % for both ranges. A calibration plot was constructed each time a set of unknown samples were analysed.

Relative Recoveries

The relative recoveries of frusemide after solid phase extraction are given in Table 2.3.

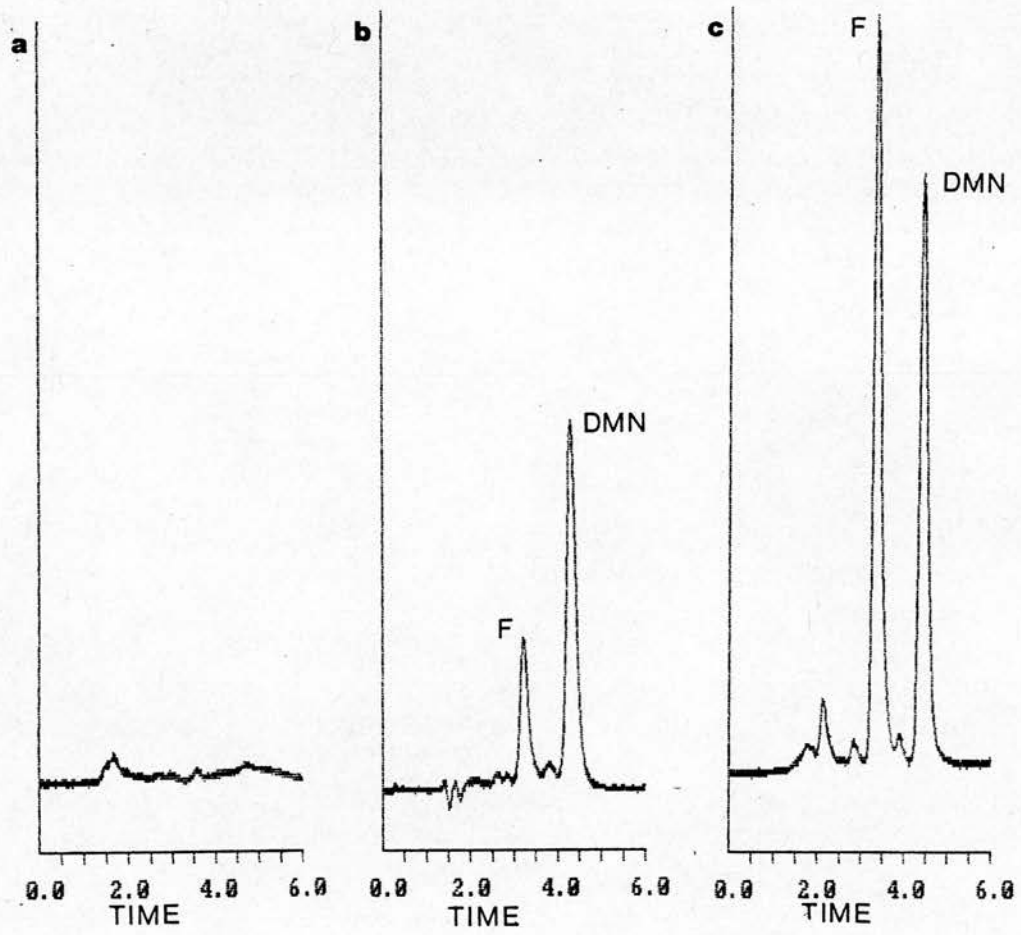


Figure 2.5. Typical chromatograms resulting from the analysis of a plasma samples collected from a healthy volunteer after receiving 40mg oral frusemide. The chromatograms show a) drug free plasma b) plasma sample containing 0.2 mg/l frusemide and the internal standard, DMN c) plasma sample containing 1.2 mg/l frusemide and DMN.

Table 2.1. Variation in plasma frusemide assay for 0.05 - 0.5 mg/l during 6 standard runs showing means, standard deviations (S.D.) and coefficients of variation (C.V.)

Plasma Frusemide Conc. (mg/l)	Peak Area Ratio (Frusemide/DMN) Run number						Mean ± S.D.	C.V. (%)
	1	2	3	4	5	6		
0.05	0.24	0.23	0.24	0.20	0.19	0.19	0.22 ± 0.02	9.09
0.1	0.36	0.38	0.42	0.38	0.39	0.38	0.39 ± 0.02	5.13
0.2	0.66	0.72	0.72	0.68	0.70	0.70	0.70 ± 0.02	2.86
0.4	1.46	1.49	1.44	1.36	1.41	1.44	1.43 ± 0.04	2.80
0.5	1.71	1.87	1.79	1.78	1.84	1.77	1.79 ± 0.05	2.79

Table 2.2. Variation in plasma frusemide assay for 0.05 - 2 mg/l during 5 standard runs showing means, standard deviations (S.D.) and coefficients of variation (C.V.)

Plasma Frusemide Conc. (mg/l)	Peak Area Ratio (Frusemide/DMN) Run number					Mean ± S.D.	C.V. (%)
	1	2	3	4	5		
0.05	0.06	0.07	0.06	0.05	0.06	0.06 ± 0.006	10.00
0.1	0.11	0.10	0.10	0.10	0.10	0.10 ± 0.004	4.00
0.5	0.48	0.46	0.51	0.47	0.45	0.47 ± 0.02	4.26
1	1.00	0.95	1.00	1.00	0.92	0.97 ± 0.03	3.09
2	2.18	2.09	2.18	2.05	2.01	2.10 ± 0.07	3.09

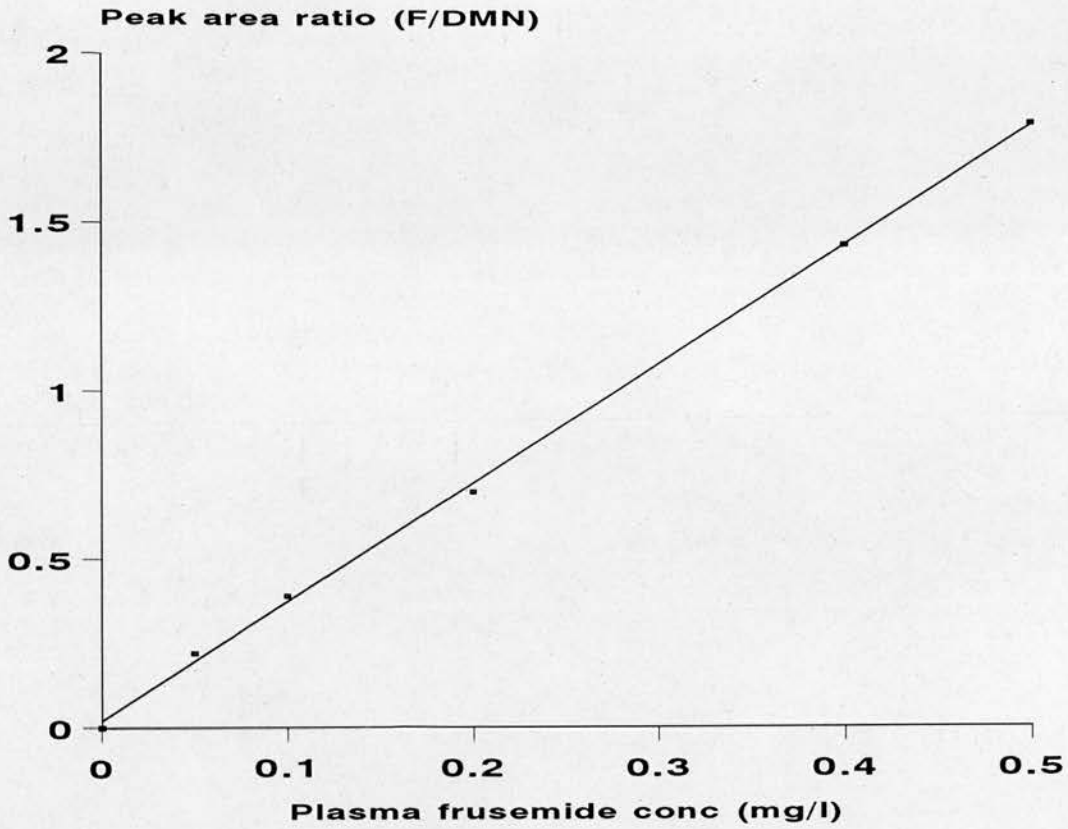


Figure 2.6. Mean standard curve obtained for 0.05 - 0.5 mg/l frusemide in plasma

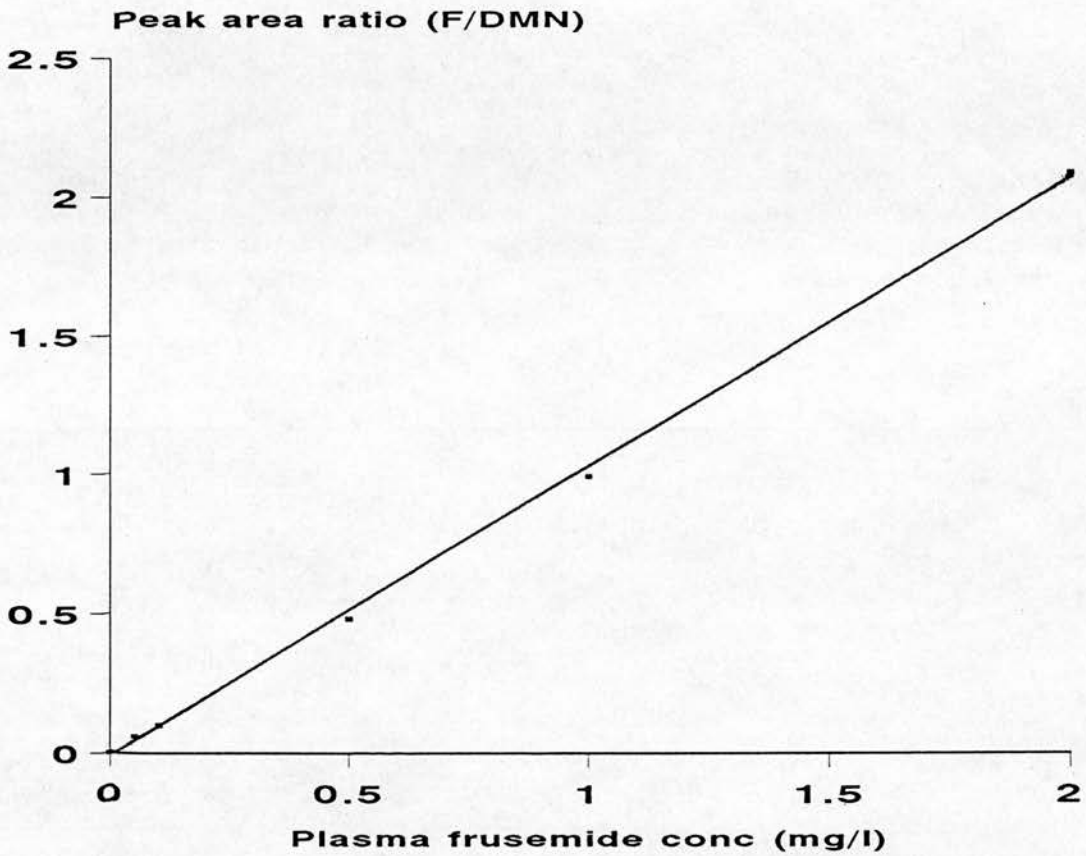


Figure 2.7. Mean standard curve obtained for 0.05 - 2 mg/l frusemide in plasma

Table 2.3. Relative recovery of frusemide from plasma after solid phase extraction. Values were obtained by comparing peak height ratios obtained after injection of extracted plasma samples containing frusemide with peak height ratios obtained after injection of non-extracted solutions of frusemide in methanol. For all concentrations, n = 5. C.V. is the coefficient of variation

Frusemide Concentration (mg/l)	Recovery (%)	C.V. (%)
0.5	101	4.9
2.0	94	1.2

Stability

When the plasma samples were left on the bench frusemide remained stable for up to 24 h. After this time a gradual reduction in peak height ratio occurred with the appearance of an additional peak running at 2.2 minutes on the chromatogram. This peak increased in size over the next few weeks. No such peak was present in the samples kept in the fridge or freezer. No instability was detected for up to 1 week in refrigerated samples and up to 8 weeks in frozen samples.

Measurement of frusemide in urine

Urine samples were injected directly into the HPLC system. No extraction procedure was thought necessary as there was no interference from endogenous peaks. 200 µl of each urine sample were pipetted into tubes together with 150 µl of 2 mg/l DMN. The tubes were whirlimixed before analysis. All apparatus and chromatographic conditions were the same as for frusemide in plasma except that the oven was set to 30° C. This provided better peak separation between frusemide and DMN in the urine.

Urine Standards

For estimating the concentration of frusemide in urine samples, standard solutions were made up with final concentrations ranging from 0.1- 5 mg/l. 150 µl of 2 mg/l DMN was used as the internal standard. 200 µl volumes of each urine standard were frozen in individual test tubes. This was the amount used during analysis. This not only reduced the time of analysis but also eliminated the repeated thaw and freeze shock to the standards. It has previously been shown that standards stored in individual containers

remain stable for longer compared with those stored in larger containers and repeatedly exposed to thaw and freeze cycles (Sood *et al.* 1987).

Stability Study

Stability studies were also performed on urine samples containing 0.1, 1 and 5 mg/l frusemide. Again samples were either left on the bench, refrigerated or frozen.

Results

Chromatograms

Chromatograms for drug free urine and for urine containing 2.5 mg/l frusemide are shown in Fig. 2.8. The total run time for each urine sample was 6 minutes in which frusemide and DMN were resolved with retention times of 3.4 and 4.8 minutes respectively.

Standard Curves

Urine standard curves were linear over the range 0.1-1 mg/l ($r = 0.9989$, $n=5$) and 1-10 mg/l ($r = 0.9998$, $n=5$). The 0.1-5 mg/l range of frusemide standards were assayed on five separate days. The results are shown in Table 2.4 and Fig. 2.9. The coefficient of variation of the assay for five replicate analyses was less than 6 %.

Stability

No instability was detected for up to 24 h in the urine samples left on the bench. Subsequently a gradual decrease in peak height ratio was observed at each concentration

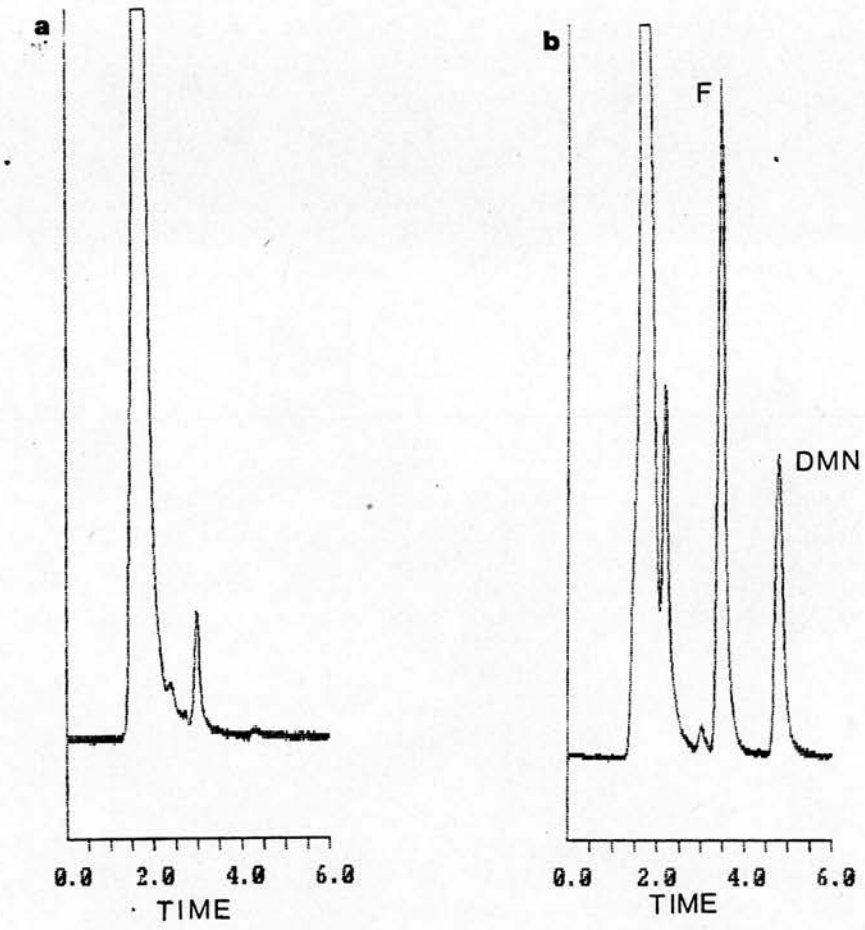


Figure 2.8. Typical chromatograms resulting from the analysis of urine samples collected from a healthy volunteer after receiving 40 mg oral frusemide. The chromatograms show a) drug free urine b) urine sample containing 2.5 mg/l frusemide collected 1 - 2 h after drug administration.

Table 2.4. Variation in urine frusemide assay for 0.1 - 5 mg/l during 5 standard runs showing means, standard deviations (S.D.) and coefficients of variation (C.V.)

Urine Frusemide Conc. (mg/l)	Peak Area Ratio (Frusemide/DMN) Run number					Mean ± S.D.	C.V. (%)
	1	2	3	4	5		
0.1	0.10	0.10	0.10	0.09	0.10	0.10 ± 0.004	4.00
0.5	0.48	0.49	0.45	0.47	0.44	0.47 ± 0.02	4.26
1.0	0.91	0.94	0.86	0.86	0.88	0.89 ± 0.03	3.37
2.0	1.74	1.82	1.74	1.77	0.61	1.74 ± 0.07	4.02
5.0	4.31	4.35	4.30	4.27	4.16	4.28 ± 0.06	1.40

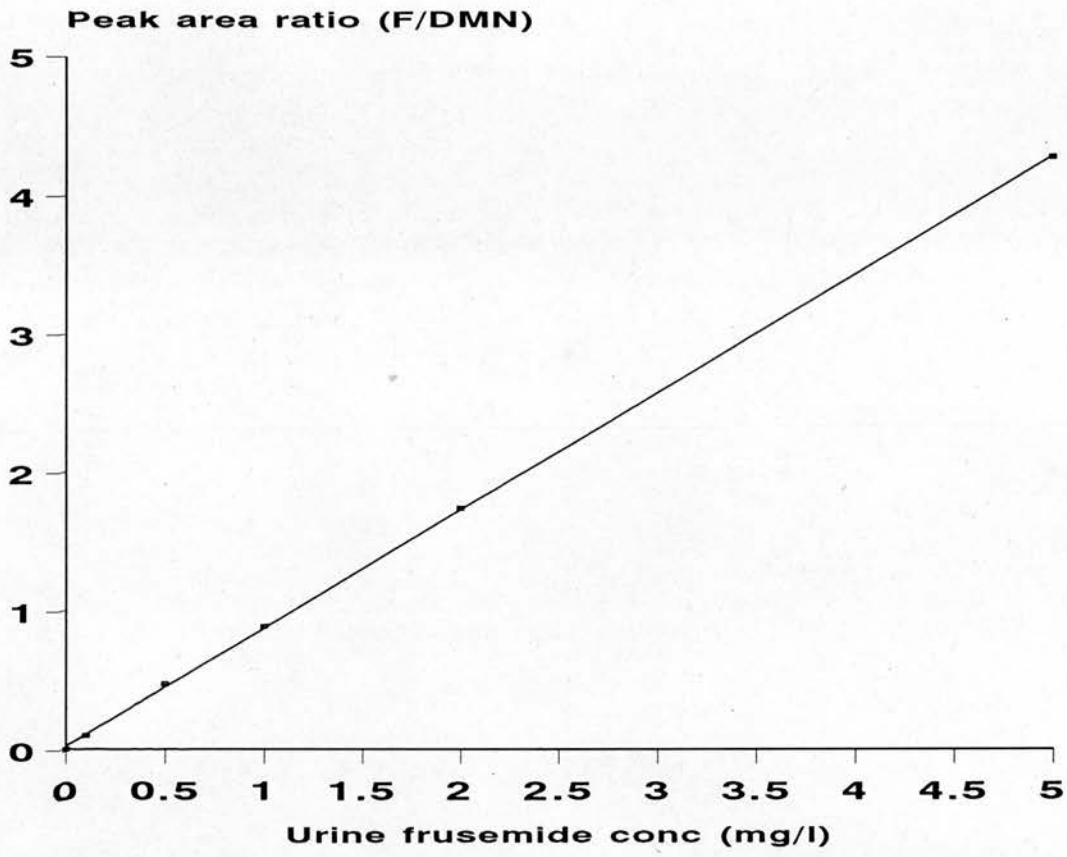


Figure 2.9. Mean standard curve obtained for 0.1 - 5 mg/l frusemide in urine

After 4 weeks no frusemide peak could be detected in either the 0.1 or 1 mg/l plasma samples. In the refrigerated and frozen samples no instability was detected for up to 4 and 10 weeks, respectively.

Measurement of bumetanide in plasma and urine by high performance liquid chromatography

Background

Gas chromatographic (Feit *et al.* 1973), radioactivity (Halladay *et al.* 1977), radioimmune (Dixon *et al.* 1976) and high performance liquid chromatographic (Ameer & Burlingame, 1988; Bökens. *et al.* 1988; .Marcantonio *et al.* 1980; Smith, 1982; Walmsley *et al.* 1981) assays are available for the determination of bumetanide in biological samples. Some of these methods have inherent disadvantages including long extraction or incubation steps, relatively large sample sizes, lack of sensitivity and dual detection for bumetanide and the internal standard. Unlike frusemide however, no stability problems have been reported for bumetanide. Bumetanide is stable for up to 24 h in plasma at acidic pH (Singh *et al.* 1989), up to 48 h in refrigerated samples and up to 6 weeks frozen (Wells *et al.* 1991).

Measurement of bumetanide in plasma

Concentrations of bumetanide in plasma were analysed using solid phase extraction and HPLC with fluorescence detection according to Wells *et al* (1991) with some modifications. It provides a sensitive, simple and relatively quick analytical method.

Materials

Bumetanide was kindly supplied by Leo Laboratories Limited (Aylesbury, England). Disodium orthophosphate and dipotassium hydrogen orthophosphate were supplied by BDH Chemicals Limited (Poole, England). Piretanide was used as the internal standard.

Plasma Standards

Stock solutions of bumetanide (0.5 mg/ml) and piretanide (1 mg/ml) were made up in methanol in standard flasks. Standards were prepared by dilution of the bumetanide stock solutions using drug free plasma. Two standard ranges were used, a low range (2.5 - 100 µg/l) and a high range (50 -500 µg/l). Both ranges contained a blank plasma standard. The corresponding internal standards used were 200 µl of 2 mg/l piretanide for the low range and 100 µl of 25 mg/l for the high range.

Sample Pretreatment

All standards and unknown samples were pretreated as follows. To each 0.2 ml of plasma the appropriate amount of piretanide was added. 0.4 ml of acetonitrile was then added to precipitate the proteins. Each sample was vortex mixed for approximately 30 seconds and centrifuged at 2000 rpm for 10 minutes. The supernatant was transferred to a 10 ml test tube and 4 ml of a 0.1 M phosphate buffer (pH5.0) was added. Bumetanide was then isolated using solid phase extraction.

Non polar Sorbent Extraction

C_{18} ODS "Bond Elut" columns were used. Prior to introducing the plasma samples, the columns were washed sequentially with 0.6 ml of acetonitrile, 2 ml of methanol and 3 ml of deionised water. The samples were then loaded onto the columns. The columns were washed with 3 ml of water. Bumetanide and piretanide were eluted with 0.3 ml of acetonitrile. The eluates were placed in a water bath at 37°C and evaporated to dryness under a stream of nitrogen. They were then reconstituted in 0.2 ml of the HPLC solvent and injected.

Apparatus and Chromatographic conditions

The same apparatus was used as described for the frusemide assay. The samples were analysed at room temperature. The luminescence spectrophotometer was set at excitation wavelength 228 nm and emission wavelength 418 nm. A Waters 5 µm "Resolve" C_{18} column was used. It was protected with a precolumn of the same packing. The mobile phase consisted of methanol, deionised water and acetic acid (66:34:1 v/v) and was delivered at a flow rate of 1.2 ml/minute.

Relative Recovery

Recovery experiments were conducted using known concentrations of bumetanide in methanol and plasma. Bumetanide in methanol (10 and 100 µg/l) was evaporated to dryness and reconstituted with 200 µl of the HPLC eluent. Piretanide was added and an aliquot injected onto the column. Plasma samples also containing 10 and 100 µg/l bumetanide were prepared and extracted as previously described. Recoveries of

bumetanide from plasma after solid phase extraction were determined by comparison of peak height ratios obtained from plasma with those of non-extracted solutions of bumetanide in methanol.

Stability

Stability studies were performed on refrigerated (4^0 C) and frozen (-20^0C) aliquots of plasma containing 20 µg/l bumetanide. Several aliquots were also left on the bench.

Results

Chromatograms

Bumetanide and piretanide in plasma were monitored over a total run time of 8 minutes and were eluted from the column with retention times of 6.5 and 5.1 minutes respectively. Fig 2.10 shows chromatograms obtained from blank plasma and plasma containing 11.7 and 99.6 µg/l bumetanide.

Standard Curves

The 2.5 - 100 µg/l and 50 - 500 µg/l bumetanide plasma standard ranges were assayed on five separate occasions. The results are shown in Tables 2.5 and 2.6 and Figs 2.11 and 2.12. The coefficient of variation of the assay was less than 10 % for the low range and less than 6 % for the high range.

Recovery

The mean relative recoveries for bumetanide plasma concentrations of 10 and 100 µg/l

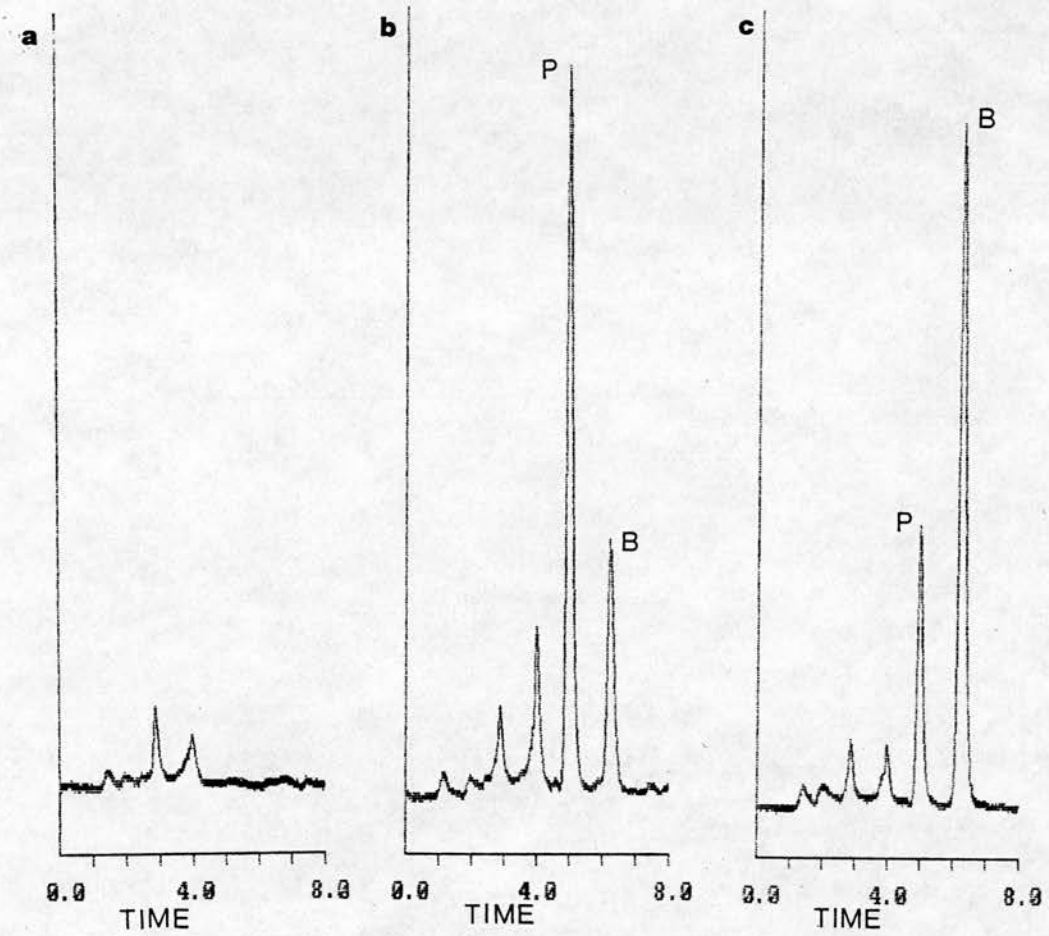


Figure 2.10. Typical chromatograms resulting from the analysis of plasma samples collected from a healthy volunteer after receiving 2 mg bumetanide. The chromatograms show a) drug free plasma b) plasma sample containing 11.7 µg/l bumetanide and the internal standard, piretanide c) plasma sample containing 99.6 µg/l bumetanide and piretanide.

Table 2.5. Variation in plasma bumetanide assay for 2.5 - 100 μg/l during 5 standard runs showing means, standard deviations (S.D.) and coefficients of variation (C.V.)

Plasma Bumetanide Conc. (μg/l)	Peak Area Ratio (Bumetanide/Piretanide) Run number					Mean ± S.D.	C.V. (%)
	1	2	3	4	5		
2.5	0.09	0.10	0.09	0.11	0.09	0.10 ± 0.008	8.00
5	0.17	0.18	0.16	0.18	0.17	0.17 ± 0.01	5.88
10	0.32	0.31	0.30	0.36	0.32	0.32 ± 0.02	6.25
20	0.62	0.63	0.57	0.69	0.68	0.64 ± 0.04	6.25
50	1.45	1.45	1.39	1.55	1.55	1.48 ± 0.06	4.05
100	2.94	2.84	2.76	3.11	2.78	2.89 ± 0.13	4.50

Table 2.6. Variation in plasma bumetanide assay for 50 - 500 µg/l during 5 standard runs showing means, standard deviations (S.D.) and coefficient of variation (C.V.)

Plasma Bumetanide Conc. (µg/l)	Peak Area Ratio (Bumetanide/Piretanide) Run number					Mean ± S.D.	C.V. (%)
	1	2	3	4	5		
50	0.26	0.25	0.28	0.28	0.28	0.27 ± 0.01	3.70
100	0.52	0.47	0.50	0.52	0.49	0.50 ± 0.02	4.00
250	1.32	1.17	1.24	1.24	1.17	1.23 ± 0.06	4.88
500	2.47	2.30	2.34	2.37	2.30	2.36 ± 0.06	2.54

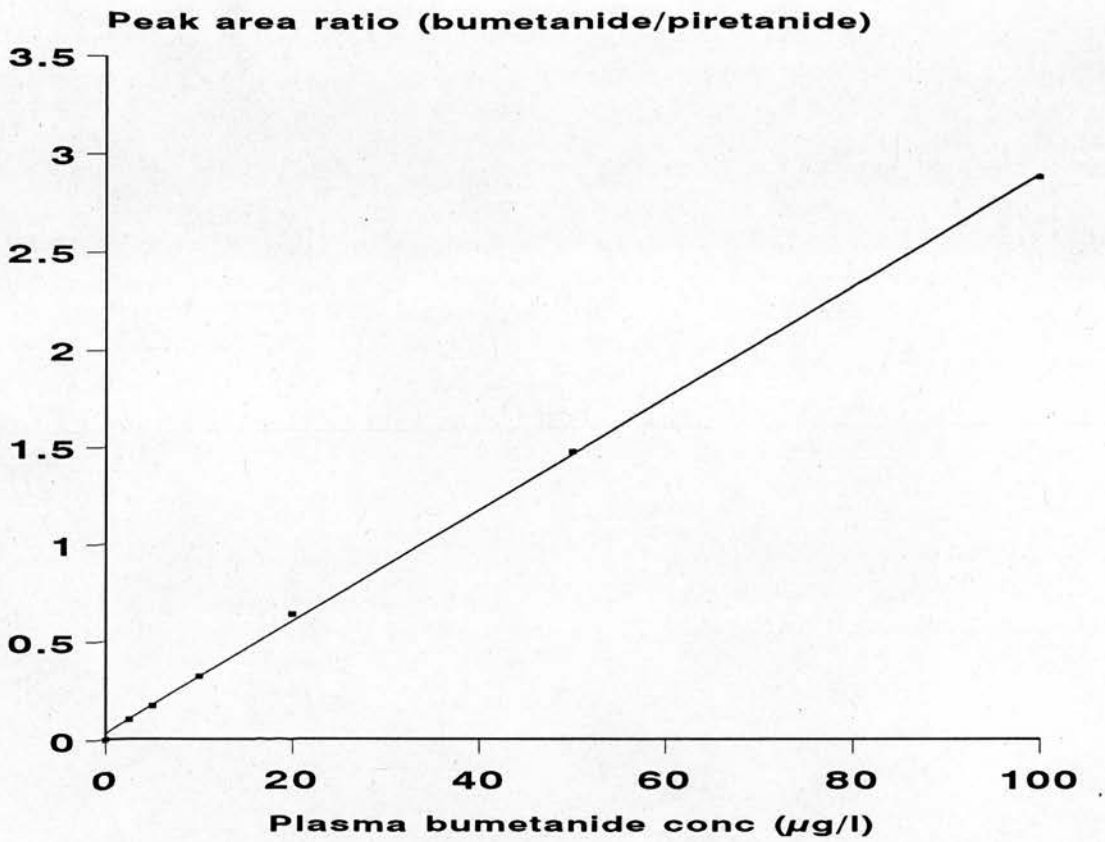


Figure 2.11. Mean standard curve obtained for 2.5 - 100 µg/l bumetanide in plasma.

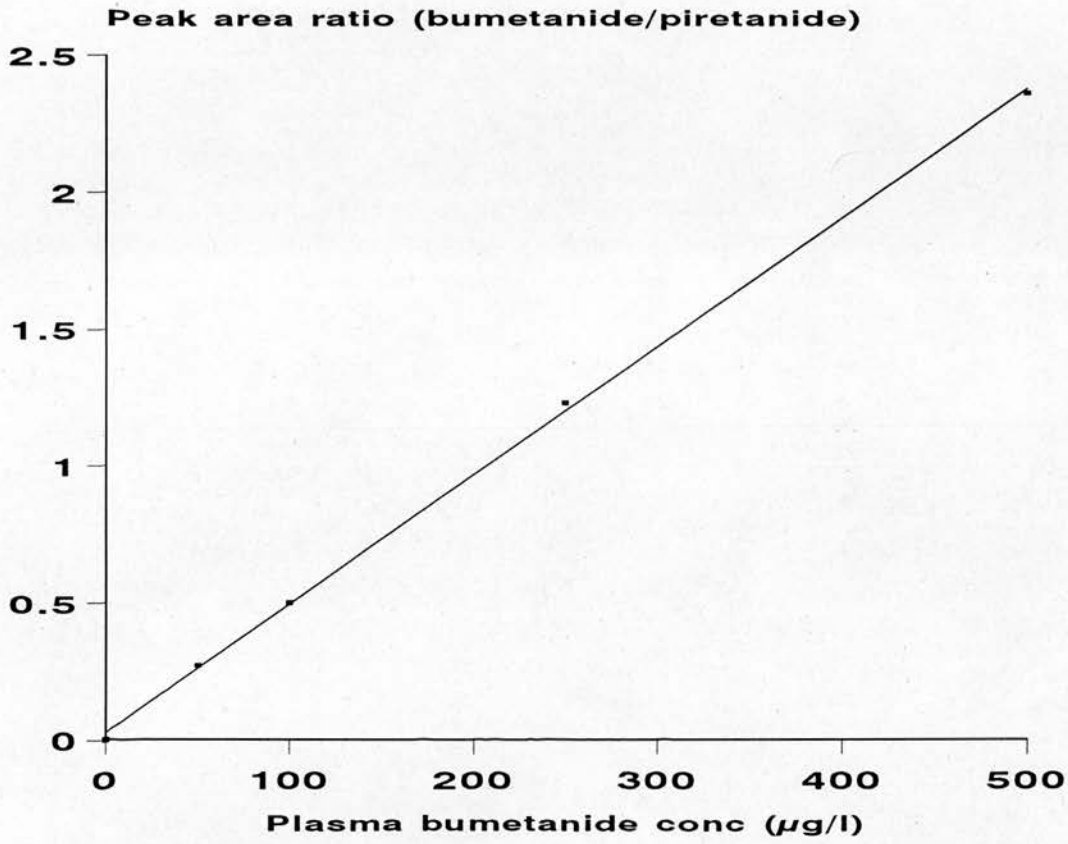


Figure 2.12. Mean standard curve obtained for 50 - 500 µg/l bumetanide in plasma.

Table 2.7. Relative recovery of bumetanide from plasma after solid phase extraction. Values were obtained by comparing peak height ratios obtained after injection of extracted plasma samples containing bumetanide with peak height ratios obtained after injection of non-extracted solutions of bumetanide in methanol. For all concentrations, n = 5. C.V. is the coefficient of variation

Bumetanide Concentration (mg/l)	Recovery (%)	C.V. (%)
10	94	3.8
100	97	2.1

are given in Table 2.7.

Stability

No instability was detected for up to 72 h in the plasma samples left on the bench. A reduction in peak height ratio then occurred and after 3 weeks no peak for bumetanide could be detected. The refrigerated and frozen samples were stable for at least 12 weeks.

Measurement of bumetanide in urine

Urine samples were introduced directly into the HPLC system. 200 µl of each urine sample were pipetted into tubes together with 200 µl of 10 mg /l piretanide. The tubes were whirlimixed before analysis. The chromatographic conditions were the same as for the plasma samples.

Urine Standards

Standard solutions of bumetanide in urine were made up with final concentrations ranging from 0.1 - 1 mg/l. The internal standard used was 200 µl of 10 mg/l piretanide.

Stability

Similar stability studies were performed on refrigerated and frozen aliquots of urine containing 0.4 mg/l bumetanide

Results

Chromatograms

The total run time for each urine sample was 9 minutes. Bumetanide and piretanide were eluted with retention times of 6.6 and 5.2 minutes respectively. Chromatograms obtained for blank urine and urine containing 0.3 mg/l bumetanide are shown in Fig2.13.

Standard Curves

The 0.1 -1 mg/l range of bumetanide urine standards were assayed on five separate occasions, the results of which are given in Table 2.8 and Fig 2.14. The coefficient of variation for the assay was less than 6 % for five replicate standard runs.

Stability

No instability was detected for up to 12 weeks in either the refrigerated or frozen samples

Measurement of urinary sodium and potassium concentrations

Sodium and potassium concentrations in urine were measured using ion specific electrodes (KNA1 Na/K Analyser, Radiometer, Copenhagen).

Measurement of plasma renin activity (PRA)

PRA was measured by radioimmunoassay of the decapeptide, angiotensin I. The assay was based on the original method developed by Haber *et al.* 1969.

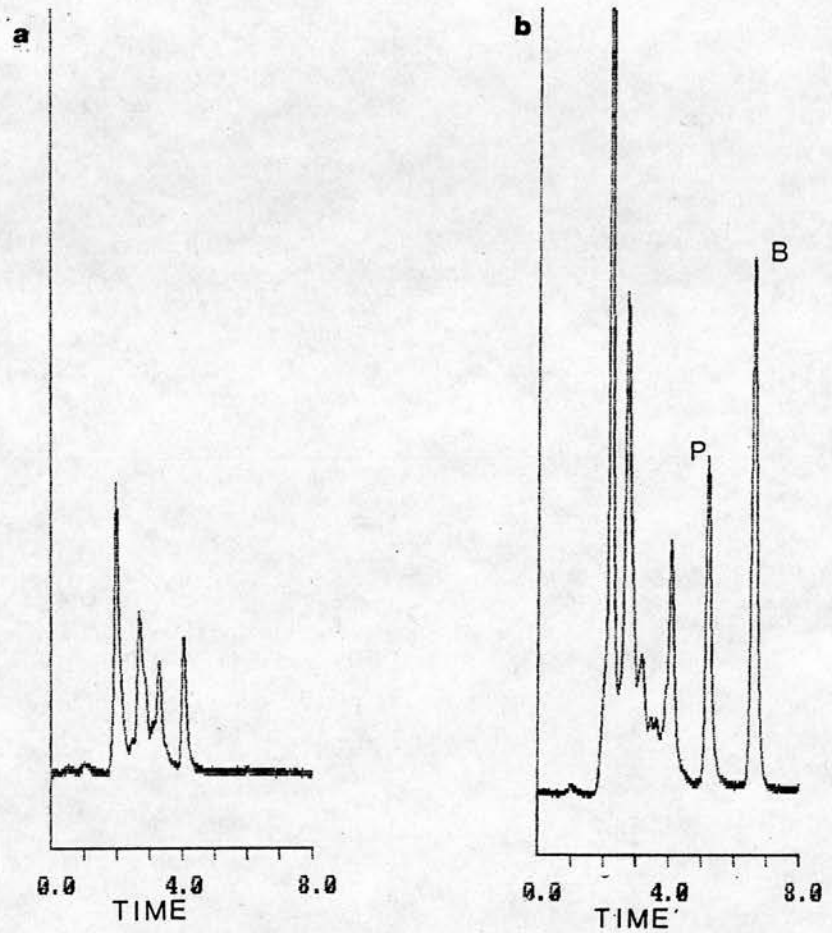


Figure 2.13. Typical chromatograms resulting from the analysis of a urine sample collected from a healthy volunteer after receiving 2mg oral bumetanide. The chromatograms show a) drug free urine b) urine sample containing 0.35 mg/l bumetanide collected 3 - 4 h after drug administration

Table 2.8. Variation in urine bumetanide assay for 0.1 - 1.0 mg/l during 5 standard runs showing means, standard deviations (S.D.) and coefficeints of variation (C.V.)

Urine Bumetanide Conc. (mg/l)	Peak Area Ratio (Bumetanide/Piretanide) Run number					Mean ± S.D.	C.V. (%)
	1	2	3	4	5		
0.1	0.48	0.48	0.46	0.5	0.48	0.48 ± 0.01	2.08
0.2	0.90	0.89	0.94	0.84	0.90	0.89 ± 0.03	3.37
0.4	1.83	1.70	1.84	1.78	1.64	1.76 ± 0.08	4.54
0.6	2.67	2.65	2.8	2.55	2.64	2.66 ± 0.08	3.01
0.8	3.58	3.36	3.77	3.70	3.52	3.59 ± 0.14	3.90
1.0	4.25	4.33	4.76	4.61	4.45	4.48 ± 0.18	4.02

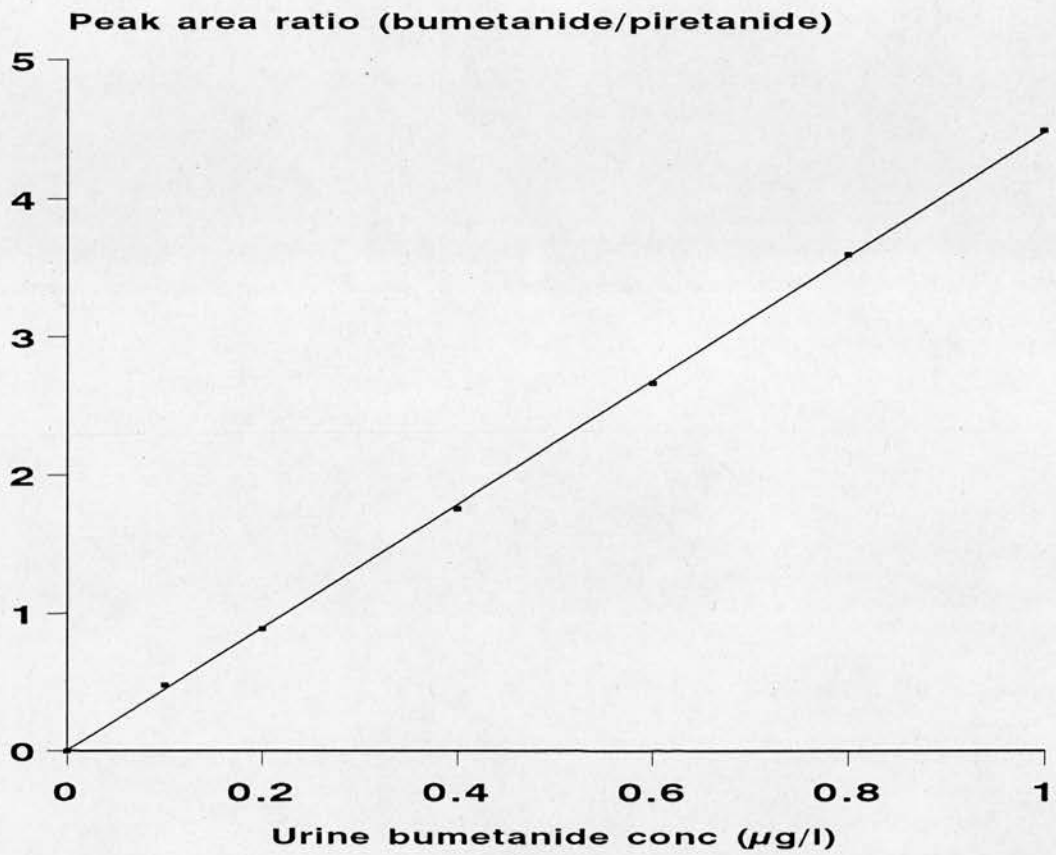


Figure 2.14. Mean standard curve obtained for 0.1 - 1 mg/l bumetanide in urine

Generation of angiotensin I

Plasma samples were thawed at room temperature. 200 µl of each plasma sample were added to test tubes followed by 40 µl of phosphate buffer (pH 5.3) and 10 µl of an inhibitor mix. The mix contained phenylmethylsulphonyl fluoride, dimercaprol and 8-hydroxyquinolone. These reagents inhibit the action of angiotensin converting enzyme and angiotensinase. The tubes were mixed and incubated at 37^0C for 1 h in a shaking water bath. After exactly 1 h the tubes were removed to a tray of ice and 1 ml of refrigerated deionised water added to arrest the reaction.

Radioimmunoassay of angiotensin I

Each plasma sample was assayed in duplicate. To each tube the following were added, 100 µl of plasma sample from above, 400 µl of 0.5 % bovine serum albumin buffer containing 0.1% lysozyme, 200 µl of angiotensin I antibody and 200 µl Iodine 125 angiotensin I label. Standard solutions containing known concentrations of angiotensin I were also prepared in 0.25 % bovine serum albumin buffer. To allow equilibration all tubes were incubated for 20-24 h at 4^0. Free angiotensin I was then separated by the addition of dextran coated charcoal. Tubes were mixed and centrifuged at 2000 rpm for 10 minutes at 4^0. Supernates were decanted into identically labelled tubes and radioactivity was counted using a 1275 Minigamma Counter (LKB, Wallac). The counter automatically constructs a standard curve and results of paired test samples are averaged and concentrations derived from the standard plot. Concentrations are expressed as pmol of angiotensin I generated $h^{-1}l^{-1}$ of plasma.

Measurement of aldosterone concentration in plasma

Aldosterone concentrations in plasma were measured using Coat-A-Count Aldosterone solid phase radioimmunoassay kits (Diagnostic Products Corporation, Los Angeles). The tubes in the kit are coated with antibody and aldosterone in the samples competes with radiolabelled aldosterone for binding to antibody sites during an incubation step. The tubes are decanted and counted. Sample concentrations are determined from a standard curve.

Section 2.2. Pharmacokinetic Analysis

Pharmacokinetics

Pharmacokinetics is the mathematical description of the time course of changes in the concentration of drugs and their metabolites in the body. It deals primarily with drug absorption, distribution and elimination. Various mathematical models have been constructed to interpret such data (Rowland and Tozer, 1980; Gibaldi and Perrier, 1982).

One compartment model

The most commonly employed approach to the pharmacokinetic characterisation of a drug is to represent the body as a system of compartments. The simplest case is the one compartment model whereby it is assumed that a drug is introduced into the circulation, distributed instantaneously throughout the body and eliminated at a rate proportional to its concentration. This is termed first order elimination and it is an exponential process (Gibaldi and Perrier, 1982).

Two compartment model

In practice, however, the one compartment model is usually inappropriate. Tissue uptake and drug distribution both take time. In such a case, following a rapid intravenous injection, two phases are seen on a log plasma concentration versus time plot (See Fig. 2.15). Initially there is a rapid decline in plasma concentrations. This fall, in addition to the process of elimination, represents drug distribution into peripheral

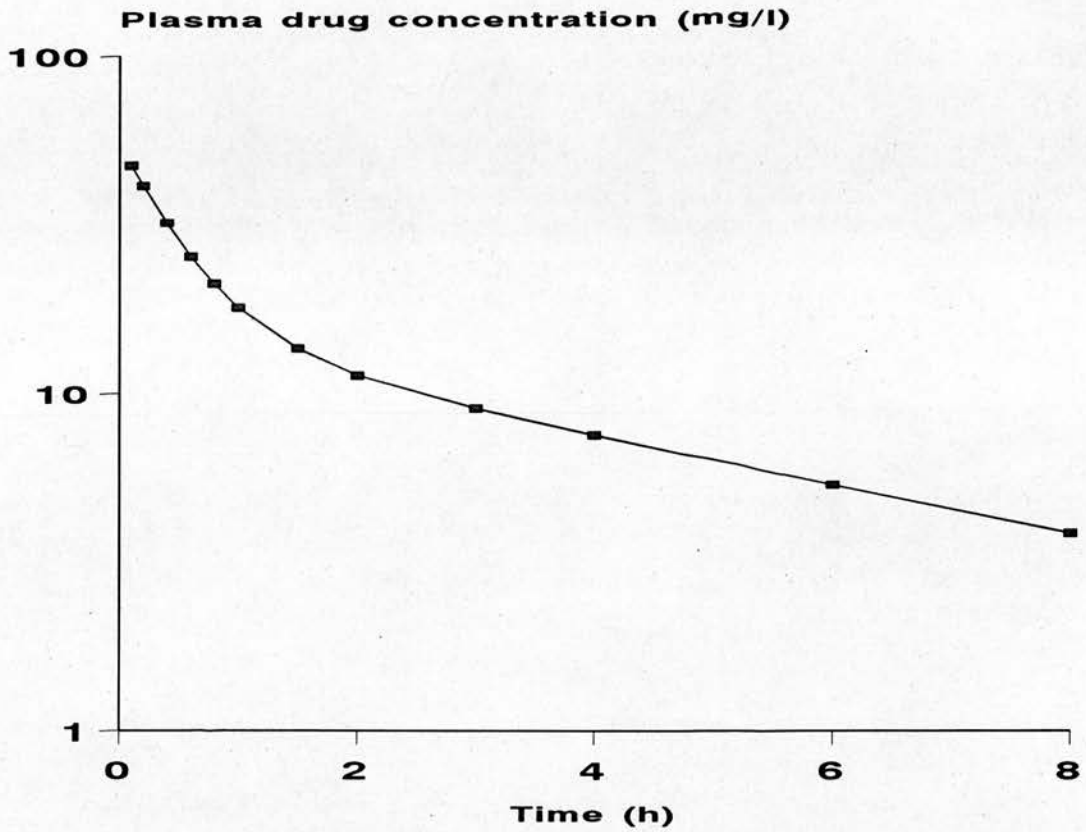


Figure 2.15. Log plasma concentration versus time plot following rapid intravenous drug administration. The early rapid decline in plasma concentrations represents the processes of drug distribution and elimination. The slower phase represents elimination only.

tissues. When the process of distribution is complete, drug concentration falls more slowly. This slower subsequent linear phase represents drug elimination only. In this model the body is therefore considered as two separate but interconnected compartments:

(a) a central compartment consisting of the circulation and well perfused organs into which the drug distributes rapidly

(b) a peripheral compartment which represents the rest of the body where drug uptake is slower (Gibaldi and Perrier, 1982)

Drug distribution and elimination therefore represent two simultaneously occurring exponential processes. These 2 phases can however be separated graphically by extrapolation and subtraction, a procedure known as the method of residuals or "curve stripping" (See Fig. 2.16). The slope of the linear elimination phase (b) is extrapolated back to zero time (B). The concentrations on this line are then subtracted from the actual plasma concentration values at each time point. The residual values generated describe the process of distribution with slope (a) and intercept A on the y-axis. The rate constants (i.e. fractional rate of change in drug concentration in unit time) for the distribution (k_d) and elimination (k_{el}) phases are given by 2.303a and 2.303b, respectively. The curve stripping procedure can also be applied following oral and intramuscular administration in order to separate out the process of absorption (See Fig. 2.17).

Certain other pharmacokinetic variables can be defined with the use of plasma concentration time curves:

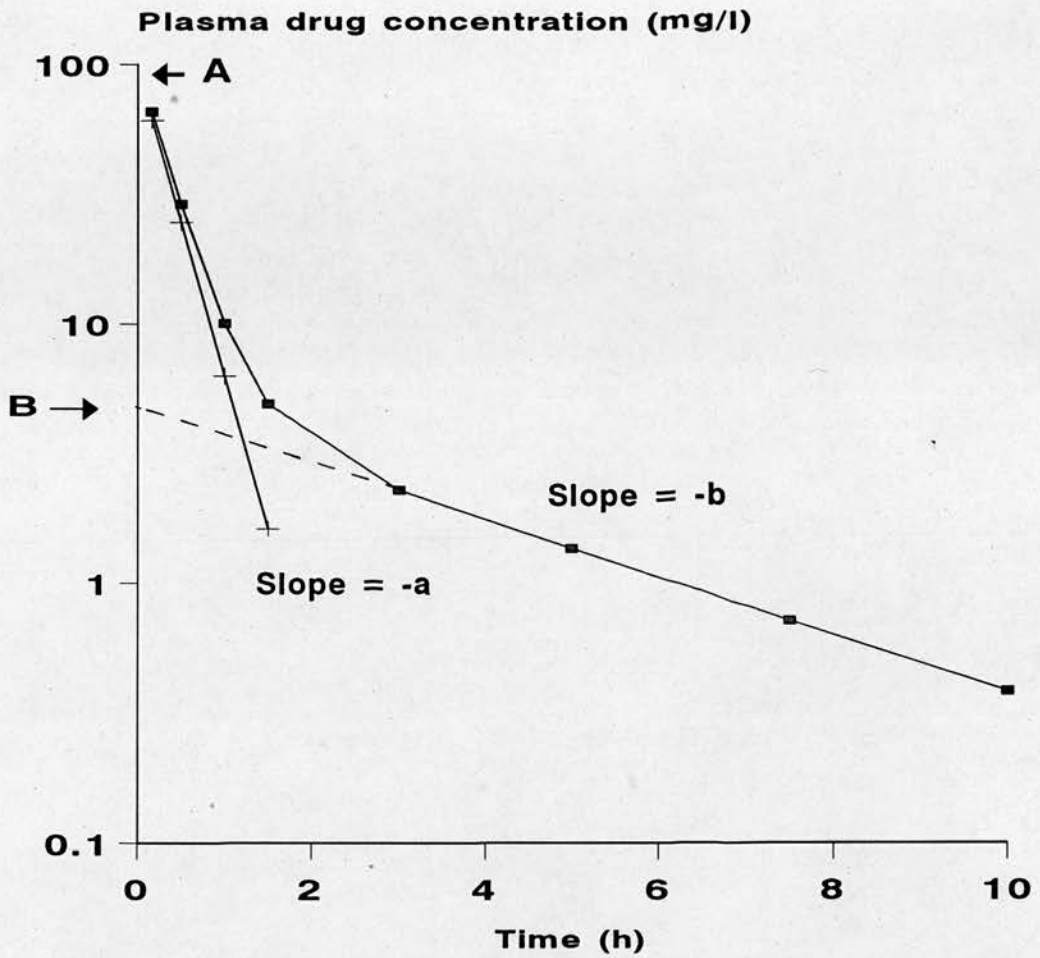


Figure 2.16. By the method of residuals, the distribution and elimination processes can be separated following intravenous administration of a drug. Elimination process has slope 'b' and intercept B. Residual values (+) describe distribution process with slope 'a' and intercept A..

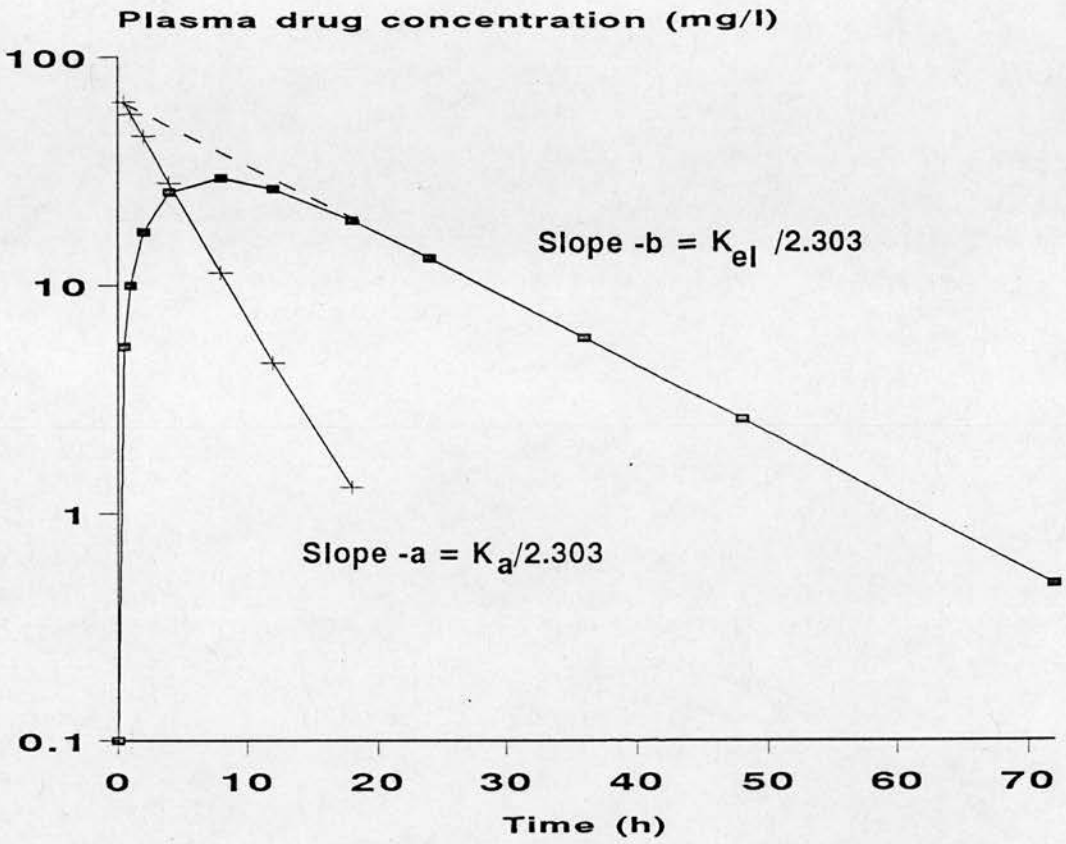


Figure 2.17 Following oral drug admnistration, the method of residuals can also be used to separate out an exponential absorption process. The terminal elimination phase with slope 'b' is extrapolated back to the y-axis. Subtraction of actual concentrations at each time point from the extrapolated values gives a series of residual values with slope 'a'. These values describe the absorption process. K_a is the rate constant for the absorption phase.

(1) *Cmax*

Maximum concentration achieved after drug administration

(2) *Tmax*

Time interval between drug administration and Cmax.

(3) *Half-life or* $t_{1/2}$

Drug half-life is defined as the time taken for the plasma drug concentration, or the amount of drug in the body to fall by one half. It is calculated from the terminal linear part of a log concentration versus time graph according to:

$$t_{1/2} = \log 2 \,/\, \text{slope (b)} \qquad \text{Equation 2.1}$$

The elimination half-life is related to the rate constant (k_{el}) by the equation (Gibaldi and Perrier, 1982):

$$t_{1/2} = \ln 2 \,/\, k_{el} \qquad \text{Equation 2.2}$$

(4) *Area under the plasma concentration time curve (AUC)*

AUC can be estimated by the trapezoidal rule illustrated in Fig. 2.18 (Gibaldi and Perrier, 1982; Notari, 1987). If a perpendicular line is drawn from each concentration point down to the x-axis, the areas produced are trapezoids. The area of each trapezoid is given by the product of the average concentration and the time interval (t)

$$\text{Area } 1 = (C_1 + C_2 / 2) \,.\, t \qquad \text{Equation 2.3.}$$

The area from the last point to infinity is calculated by:

$$\text{Area}_{t\text{-}\infty} = C_t / k_{el} \qquad \text{Equation 2.4.,}$$

where C_t = concentration at last time point, Kel = elimination rate constant.

Total AUC is therefore given by:

$$AUC_{0\text{-}\infty} = \text{sum of individual areas} + C_t / k_{el} \qquad \text{Equation 2.5}$$

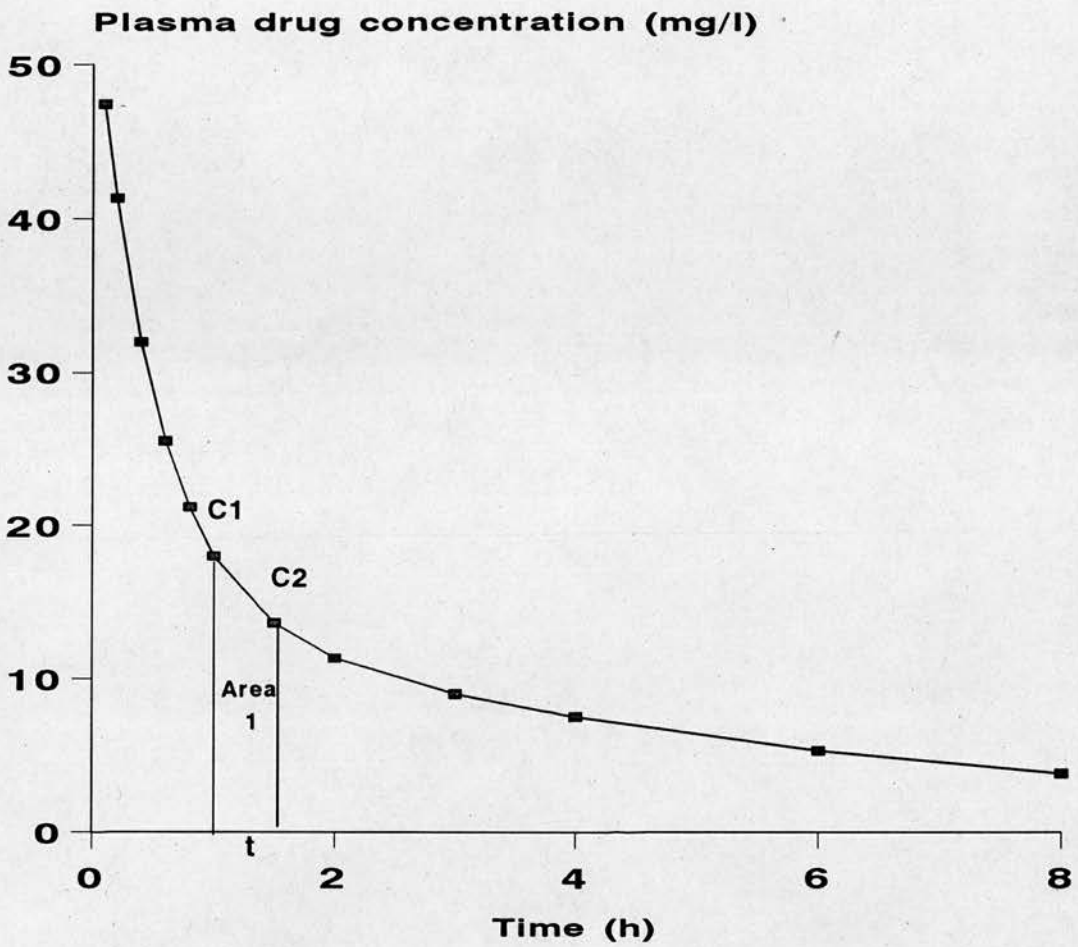


Figure 2.18. Plot of plasma concentration-time data following intravenous drug administration. Area 1, which is a trapezoid, can be calculated as a product of (C1 + C2 / 2) and t, the time interval. Summing all areas will give the AUC.

(5) *Volume of distribution (Vd)*

The apparent volume into which a drug distributes in the body at equilibrium is called the volume of distribution. In a one compartment model it can be calculated by the expression:

$$Vd = Dose / C_o \qquad \text{Equation 2.6,}$$

where Dose = actual dose of drug administered, C_o = theoretical plasma concentration at time zero.

C_o is obtained by extrapolating the linear part of the log concentration time plot back to the y-axis. However this is only true for a one compartment model with instantaneous drug distribution and in other circumstances volume may be overestimated. A better model independent way to determine Vd is to use the relationship:

$$Vd = Dose / Area . k_{el} \qquad \text{Equation 2.7,}$$

where Dose = fraction of dose which reaches the circulation intact, Area = area under the plasma concentration time curve, k_{el} = elimination rate constant.

This expression, sometimes known as Vd_{area} can therefore be used to describe the volume of distribution for drugs which follow multiexponential decay (Gibaldi and Perrier, 1982; Rowland and Tozer, 1980).

(6) *Clearance (CL)*

Clearance of a drug is defined as the volume of blood from which all drug would appear to be removed per unit time. Total body clearance is equal to the sum of all individual clearances which comprise drug elimination and following parenteral administration is given by:

$$CL = \text{Intravenous Dose} / AUC_{0-\infty} \qquad \text{Equation 2.8.}$$

Total clearance can be divided into renal (CL_R) and non renal (CL_{NR}) clearances. By adapting the above definition renal clearance can be defined as the fraction of the apparent volume of distribution from which a drug is removed by renal excretion per unit time. It can be estimated by:

$$CL_R = \text{Amount excreted in time t} / AUC_{0-t} \qquad \text{Equation 2.9.}$$

Non renal clearance is the difference between total and renal clearance.

(7) *Bioavailability (F)*

This is defined as the extent and rate of entry of an administered drug into the systemic circulation intact (Rowland and Tozer, 1980). It can be determined either from:

$$F = AUC_{0-\infty}(\text{extravascular}) / AUC_{0-\infty}(\text{i.v.}) \qquad \text{Equation 2.10,}$$

or

$$F = Ae(\text{extravascular}) / Ae(\text{intravenous}) \qquad \text{Equation 2.11,}$$

where Ae = total amount of drug excreted.

Calculation of all the variables described above is facilitated by use of computer software designed specifically for the treatment of pharmacokinetic data.

"Siphar"

"Siphar" (Centre d'Etudes et de Recherché en Statistiques et Informatique Médicales, Creteil cedex - France) is an interactive computer program which allows different kinetic models to be fitted to the same set of data. Experimental data from a study are first entered into a database. Using a "peeling" algorithm the user can then display graphically a semi-log plot of drug concentration versus time and decide visually the

number of exponentials suitable to describe the data. A theoretical curve is then superimposed onto the experimental data points. The peeling algorithm therefore provides an initial estimate of parameters for the chosen exponential model. In order to minimise the difference between the theoretical curve and the observed data these unrefined parameters can then be iteratively improved by using different weighting factors ($1, 1/y, 1/y^2$). $1/y$ and $1/y^2$ give increasingly less weight to lower concentration of drug where analytical precision is lowest.

Several criteria can be used to evaluate the goodness of fit of a model to experimental data: (a) residuals (differences between calculated and observed concentrations). These should be randomly distributed according to the particular model used; (b) coefficient of variation of each parameter. If the coefficient of variation is greater than 20-30 %, the lack of accuracy can be considered too large. The model and/or the computed algorithm must then be revised.

From the derived "best fit" model the program calculates elimination half life, absorption, distribution and elimination rate constants and the $AUC_{0-\infty}$. Volume of distribution (Vd), total clearance (CL) and renal clearance (CL_R) were calculated using Equations 2.7, 2.8 and 2.9.

In the following studies frusemide and bumetanide were administered orally and by intravenous infusion over 5 or 10 minutes. Resulting plasma concentration time curves were fitted using "Siphar".

Plasma concentration time curves following oral frusemide and bumetanide

administration were fitted to a one compartment model with 2 phases according to:

$$C_t = -Ae^{-k_a t} + Be^{-k_{el} t} \qquad \text{Equation 2.12}$$

where C_t is the plasma concentration at time 't', A and B are the intercepts on the y-axis representing absorption and elimination, respectively and k_a and k_{el} are the absorption and elimination rate constants of the drug (Gibaldi and Perrier, 1982).

When the drugs were administered by intravenous infusion, the plasma concentration time curves were best described by a two compartment model. The concentrations in plasma as a function of time 't' were calculated by:

$$C_t = D(1-e^{-k_d t})/k_d T + B(1-e^{-k_{el} t})/k_{el} T \qquad \text{Equation 2.13}$$

where $t < T$ (T = duration of the infusion) and

$$C_t = D(1-e^{-k_d t})e^{-k_d (t-T)}/k_d T + B(1-e^{-k_{el} t})e^{-k_{el} (t-T)}/k_{el} T \qquad \text{Equation 2.14}$$

where t > T, D is the intercept on the y-axis representing distribution and k_d is the rate constant of distribution (Notari, 1987).

CHAPTER 3

EFFECTS OF FOOD ON THE BIOAVAILABILITY OF FRUSEMIDE

Section 3.1. Introduction

Frusemide is a weak acid which is absorbed incompletely from the gastrointestinal tract and there have been reports of considerable intra and interindividual variability in its bioavailability (Ponto and Schoenwald, 1990; Smith *et al.* 1980; Waller *et al.* 1982). The extent of absorption has previously been shown to be inconsistently influenced by the presence of food.

Preliminary experiments in nonfasted intact rats suggested that oral absorption of frusemide with food in the stomach was slower but more complete than in the absence of food (Chungi *et al.* 1979). These results have not been substantiated in man. Kelly *et al* (1973) and Hammarlund *et al* (1984) did not find statistically significant differences in the extent of absorption of frusemide when given with food compared with the fasting state. Hammarlund *et al* (1984) did find that food intake significantly delayed absorption by approximately 60 minutes. On the other hand Beermann and Midskov (1986) found a 30% decrease in bioavailability when frusemide was administered after breakfast. This produced a corresponding reduction in the total diuretic effect.

The aim of this study was to obtain additional information about the individual variation and the effects of food on frusemide absorption.

Section 3.2. Methods

Subjects

Eight healthy male volunteers aged 21-38 years (28 ± 5 yr) and weighing 51 to 82 kg

(70 ± 10 kg) took part in the study. They were healthy according to medical history, clinical examination and haematological and biochemical tests. All volunteers were informed of the nature of the study and each gave written informed consent. The study was approved by the Lothian Health Board Healthy Volunteers Studies Ethics of Medical Research Sub-Committee. The volunteers were asked to:

(1) avoid alcohol for 24 h before each study day
(2) avoid taking any other medication for 1 week prior to and throughout the study
(3) fast from 22.00 h the night before each study day

Procedure

An open randomised design was used. Each volunteer was studied on 3 separate occasions at least one week apart. On each occasion they attended the clinical investigation room at approximately 8 am. On arrival, an intravenous cannula (Venflon 2, 18G) was placed into a vein in each forearm , one for the administration of drug and fluid and one for blood sampling. After emptying their bladders the volunteers then received one of the following:

(1) Intravenous frusemide (constant infusion over 5 min.) - 40 mg
(2) Oral frusemide solution - 40 mg (Lasix injection solution 10mg/ml)
(3) Oral frusemide solution after breakfast - 40 mg (Lasix injection solution 10mg/ml)

The oral doses of frusemide were taken in 100 ml of orange squash (Kia-Ora) and washed down with 100 ml of water. The breakfast consisted of fruit juice (200 ml), scrambled egg, cornflakes with milk and 2 slices of toast with butter and jam. Subjects receiving the oral dose took it immediately after breakfast.

The volunteers remained supine throughout the study and had nothing to eat or drink up to 5 h after dosing. A light lunch was provided between 5 - 6 h. To compensate for fluid loss, 500 ml of 0.9 % sodium chloride solution was administered intravenously over the first hour using an "IMED 960" volumetric infusion pump. For the remainder of the study hourly urine volumes were replaced by similar volumes of 5 % dextrose, again given intravenously.

Venous blood samples (10 ml) were taken just before dosing and at 3, 5, 10, 15, 30, 45, 60, 90, 120 min. and hourly for the next 6 h after administration of the intravenous dose. After the oral dose samples were taken at 0, 10, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180 min. and hourly for the next 5 h. All blood samples were collected in lithium heparin tubes and centrifuged at 3000 rpm for 15 min. Plasma was stored at approximately -20^{0}C.

Urine was collected at 1, 2, 3, 4, 5, 6 and 8 h after dosing. The volunteers also completed an 8- 24 h urine collection at home. Urine volumes were recorded and aliquots stored at -20^{0}C until analysis.

Analysis of samples

Concentrations of frusemide in plasma and urine were measured by high performance liquid chromatography with fluorescence detection as described in Chapter 2. Urinary sodium and potassium concentrations were analysed by ion specific electrodes.

Intravenous dose

Plasma concentration time curves after intravenous administration of frusemide were fitted to the biexponential Equation 2.14, using the "Siphar" curve fitting and modelling program.

Oral Dose

Following oral administration of frusemide in the fasting state plasma concentration time curves were fitted to a one compartment model with 2 phases according to Equation 2.12. Plasma concentration time curves following oral administration with food could not be fitted to the model by computer because of irregular absorption. The $AUC_{0\text{-}8h}$ for these data were calculated using the trapezoidal rule. The area beyond the last measured concentration to infinity was estimated from the elimination rate constant obtained after intravenous administration according to:

$$AUC_{t\text{-}\infty} = C_t / K_{el},$$

where C_t is the last measured concentration-time point and K_{el} is the intravenous elimination rate constant. It was assumed that the disposition kinetics of the drug did not change beyond this point.

The potential error in this estimation is quite small inasmuch as the extrapolated AUC values represent only 1.6 - 8.1 % (4.6 ± 2.4 %) of the total AUC values.

Statistics

All data are expressed as means ± standard deviations (s.d.). Statistical differences between doses were determined using Analysis of Variance. P values of <0.05 were accepted as significant.

Section 3.3 Results

Intravenous Administration

The mean plasma concentration versus time plot is shown in Fig 3.1 and the resulting pharmacokinetic parameters in Table 3.1. The mean biological half life calculated from the terminal elimination phase was 96 ± 40 min.

The total plasma clearance (CL) of frusemide was 147.7 ± 28.3 ml/min. Of this the renal clearance (CL_R) was 99.1 ± 17.9 ml/min and the non renal clearance (CL_{NR}) was 48.6 ± 12.7 ml/min (See Table 3.2). Renal elimination therefore contributed approximately 67 % to the overall elimination of frusemide. The volume of distribution was found to be 0.27 ± 0.10 L/kg.

The amount of frusemide excreted unchanged in the urine over 24 h was 26.9 ± 1.38 mg, which corresponds to 67.2 ± 3.4 % of the dose.

Oral administration

Mean plasma concentration time curves for oral doses given with and without food are shown in Fig 3.2 and individual plasma concentration time curves are depicted in Fig 3.3.

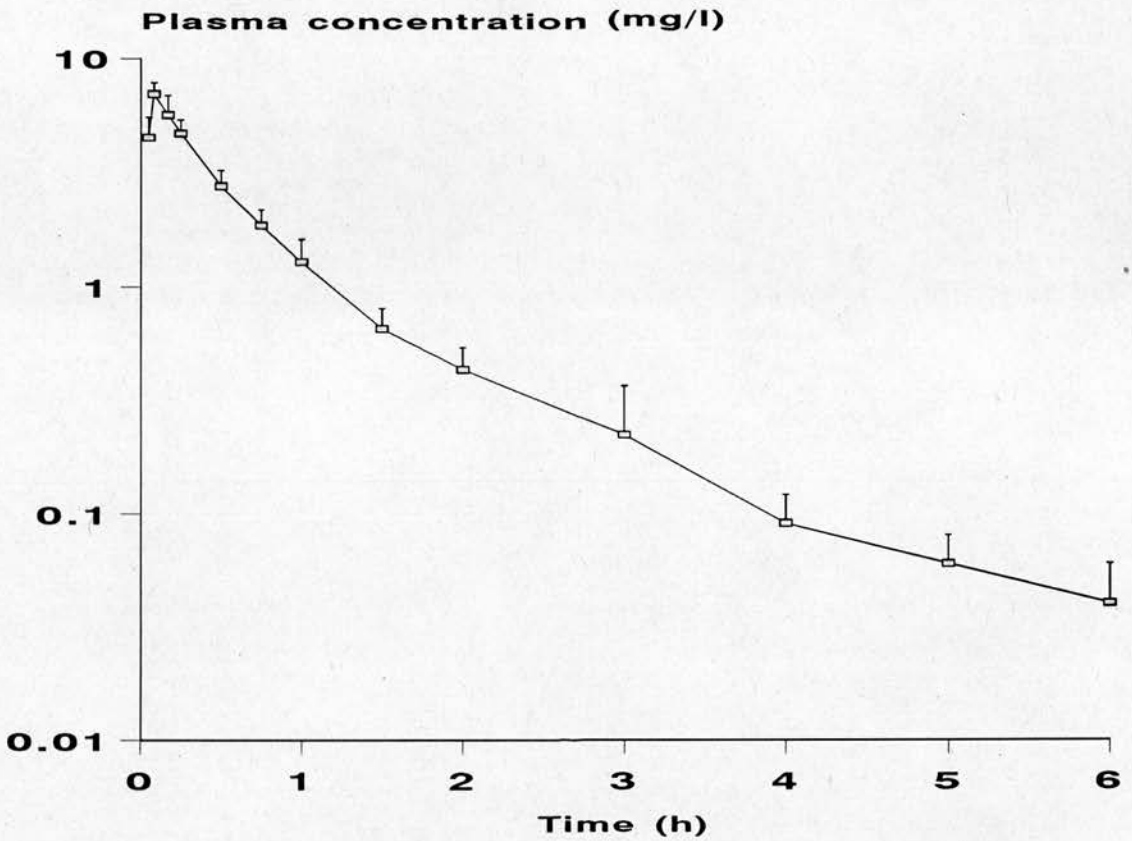


Figure 3.1. Mean plasma concentrations following intravenous administration of 40mg frusemide over 5 minutes to 8 healthy volunteers. Bars = ± s.d.

Table 3.1. Pharmacokinetic data of frusemide 40mg given intravenously (mean ± s.d.)

Intercept/ Constant	Parameter estimate ± s.d.	Half-life (min)
D (mg/l)	6.79 ± 0.86	17 ± 7.5
k_d (h^{-1})	2.88 ± 1.93	
B(mg/l)	0.97 ± 0.91	96 ± 40
k_{el} (h^{-1})	0.51 ± 0.22	

Table 3.2. Total plasma clearance (CL), renal clearance (CL_r) and volume of distribution (Vd) for frusemide after intravenous and oral administration of 40mg.

Subject	CL (ml/min)	CL_r (ml/min)			Vd_{area}
	i.v. dose	i.v. dose	oral fasting	oral with food	(L/kg) (i.v. dose)
1	123.5	82.7	86.8	103.6	0.14
2	146.8	98.8	80.8	72.2	0.26
3	207.7	128.8	157.3	163.0	0.25
4	165.8	120.2	112.8	95.8	0.16
5	148.8	105.7	53.7	97.3	0.28
6	120.8	81.2	65.2	135.5	0.31
7	133.7	90.2	83.2	66.7	0.27
8	134.3	83.8	83.8	142.3	0.49
Mean	147.7	99.1	90.4	109.6	0.27
±s.d.	28.3	17.9	29.9	32.0	0.10

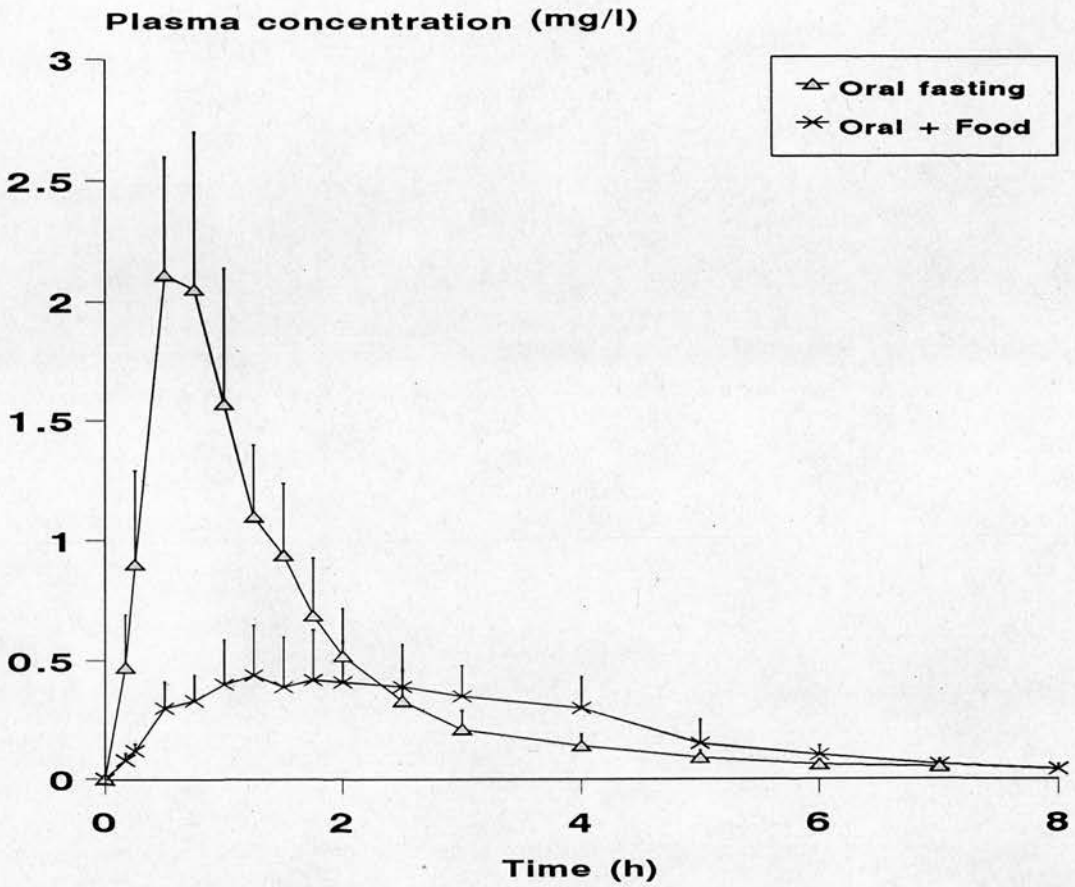


Figure 3.2. Mean plasma concentrations following oral administration of 40mg frusemide with and without food to 8 healthy volunteers. Bars = ± s.d.

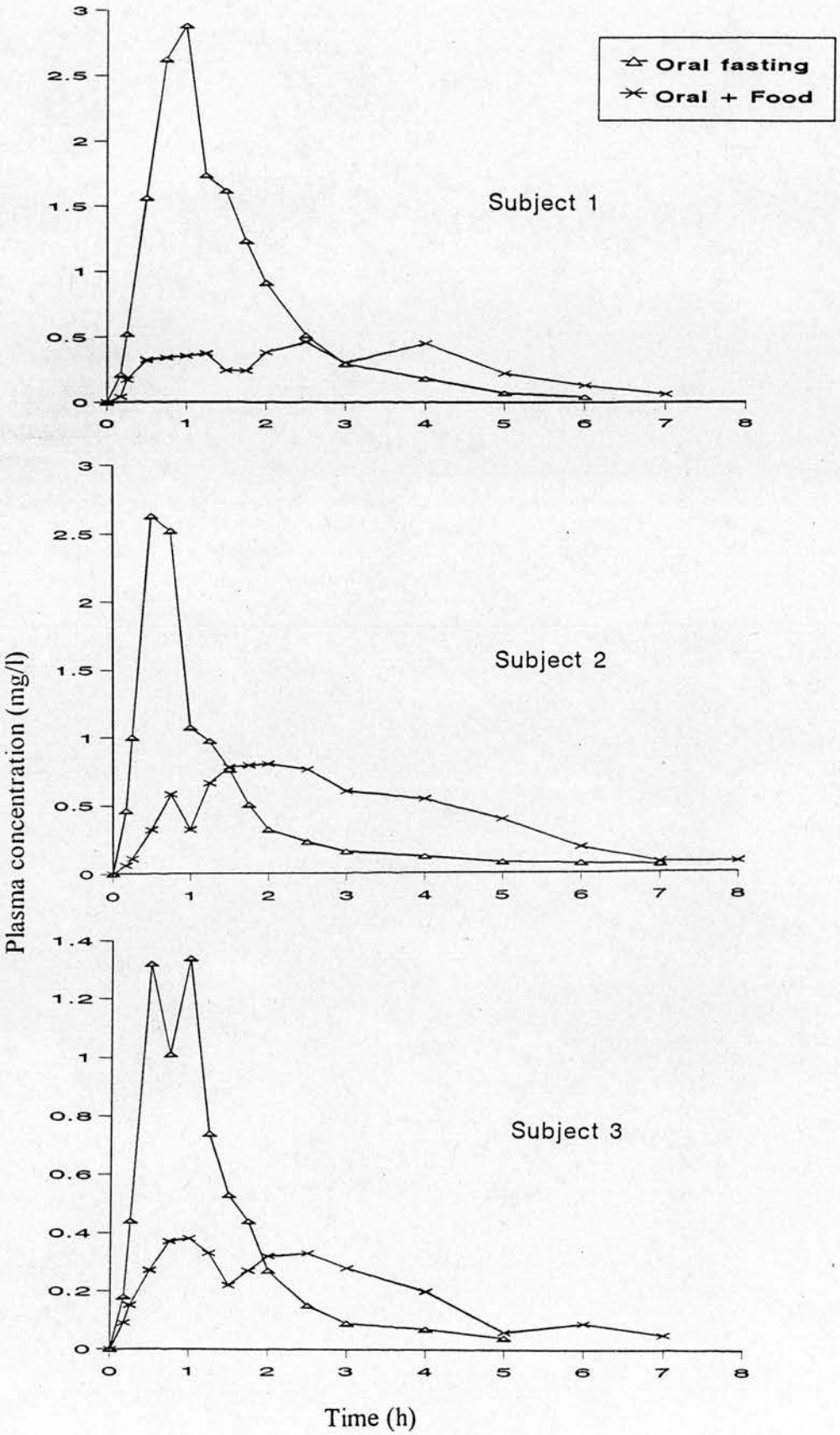


Figure 3.3. Individual plasma concentration versus time curves following oral administration of 40mg frusemide with and without food.

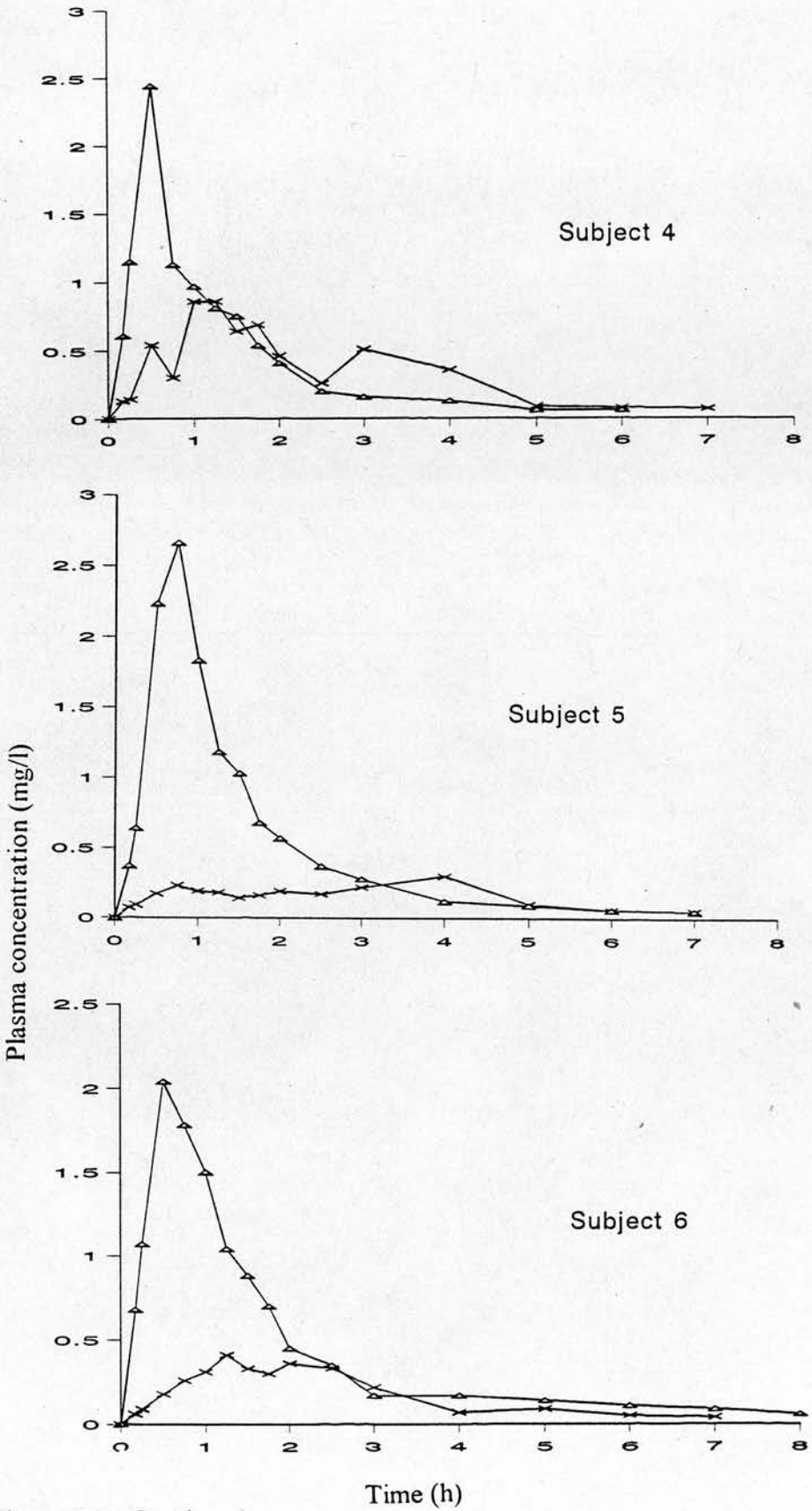


Figure 3.3. Continued.

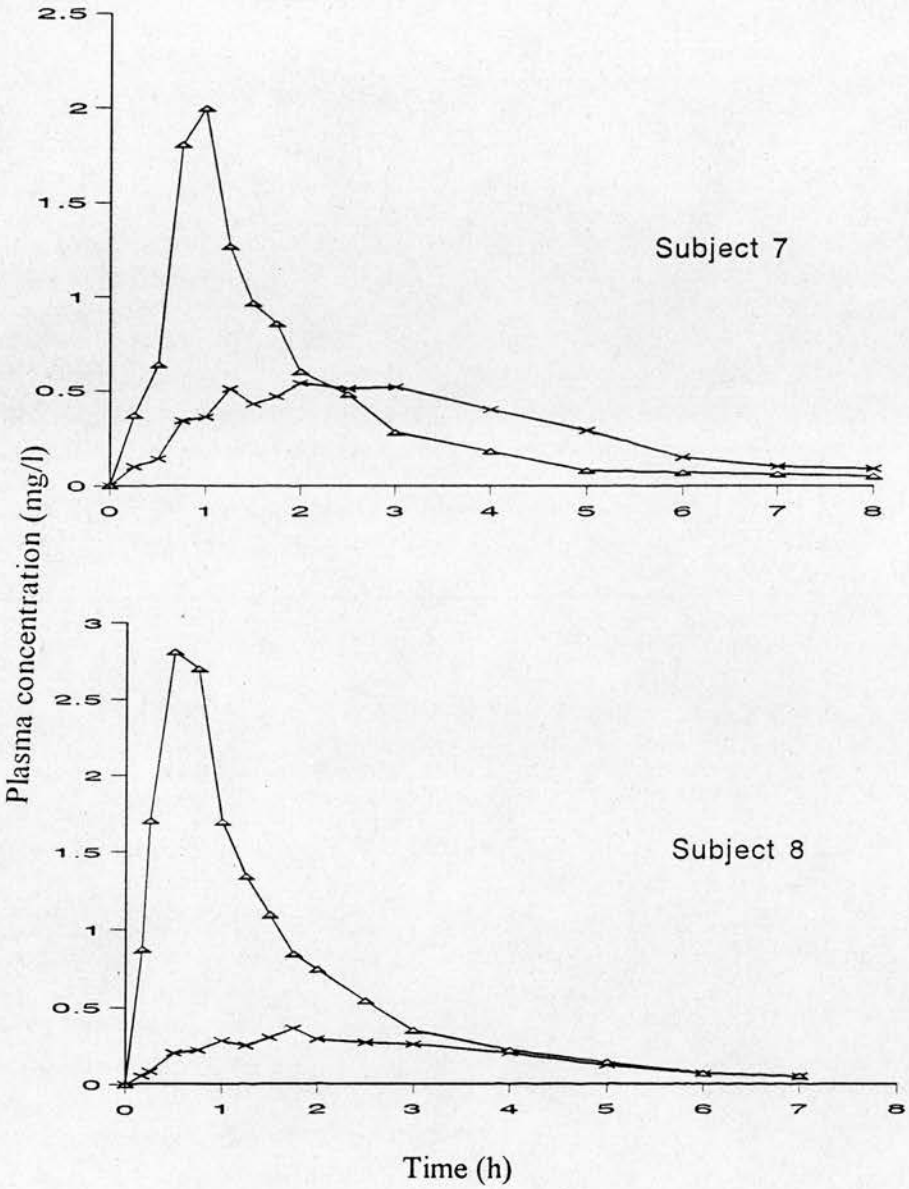


Figure 3.3. Continued

The presence of food changed the shapes of the curves for all individuals. The mean peak plasma concentration was reduced from 2.35 ± 0.49 to 0.51 ± 0.19 mg/l ($P<0.001$) and the time to peak concentration was significantly delayed (0.69 ± 0.21 to 1.91 ± 0.93 h, $P<0.01$). The $AUC_{0-\infty}$ averaged 3.54 ± 0.82 mg.h/l when frusemide was administered in the fasting state compared with 1.95 ± 0.72 mg.h/l when administered with food (Table 3.3). The difference was significant, $P<0.01$.

Bioavailability

Oral bioavailabilities of frusemide administered with and without food were calculated according to Equation 2.10 and the results are given in Table 3.4. Bioavailability was significantly reduced from 75.6 ± 10.6 % fasting to 43.2 ± 16.8 % when administered with food ($P<0.01$).

Renal elimination

The urinary recoveries of frusemide following oral and intravenous administration are shown in Fig 3.4. In the first 2 h, urinary recovery of the drug was significantly higher after the intravenous dose compared with both the oral doses. In the same period frusemide recovery was also significantly higher on comparing the oral fasting dose to the oral dose given with food. From 2-8 h however, urinary recovery of frusemide was significantly higher for the oral dose administered with food. The mean 24 h recoveries for each dose are shown in Fig 3.5. A significant decrease in the amount of frusemide eliminated in the urine can be seen on comparing fasting (45.4 ± 7.9 %) and non-fasting (29.6 ± 6.1 %) states. Both recoveries were significantly lower than the intravenous

Table 3.3. Peak concentrations (Cmax), time to peak concentrations (Tmax) and area under the plasma concentration time curves ($AUC_{0-\infty}$) following oral administration of 40 mg frusemide to 8 healthy volunteers with and without food.

Subject	Cmax (mg/l		Tmax (h)		$AUC_{0-\infty}$ (mg.h/l)	
	Without food	With food	Without food	With food	Without food	With food
1	2.88	0.46	1.00	2.50	4.33	2.00
2	2.63	0.80	0.50	2.00	3.98	3.43
3	1.34	0.38	1.00	1.00	1.93	1.47
4	2.44	0.86	0.50	1.00	2.69	2.36
5	2.66	0.30	0.75	4.00	3.81	1.22
6	2.04	0.41	0.50	1.25	4.18	1.26
7	2.00	0.54	0.75	1.75	3.09	2.47
8	2.81	0.36	0.50	1.75	4.33	1.42
Mean	2.35	0.51	0.69	1.91	3.54	1.95
± s.d.	0.49	0.19	0.21	0.93	0.82	0.72
P	<0.001		<0.01		<0.01	

Table 3.4. Bioavailability of 40mg oral frusemide administered with and without food to 8 healthy volunteers.

Subject	$AUC_{0-\infty}$ (mg.h/l) iv.	Bioavailability (%)	
		Without food	With food
1	5.40	80.2	37.0
2	4.54	87.7	75.6
3	3.21	60.1	45.8
4	4.02	66.9	58.7
5	4.48	85.0	27.2
6	5.52	75.7	22.8
7	4.99	61.9	49.5
8	4.96	87.3	28.6
Mean	4.64	75.6	43.2
±s.d.	0.71	10.6	16.8
P		<0.01	

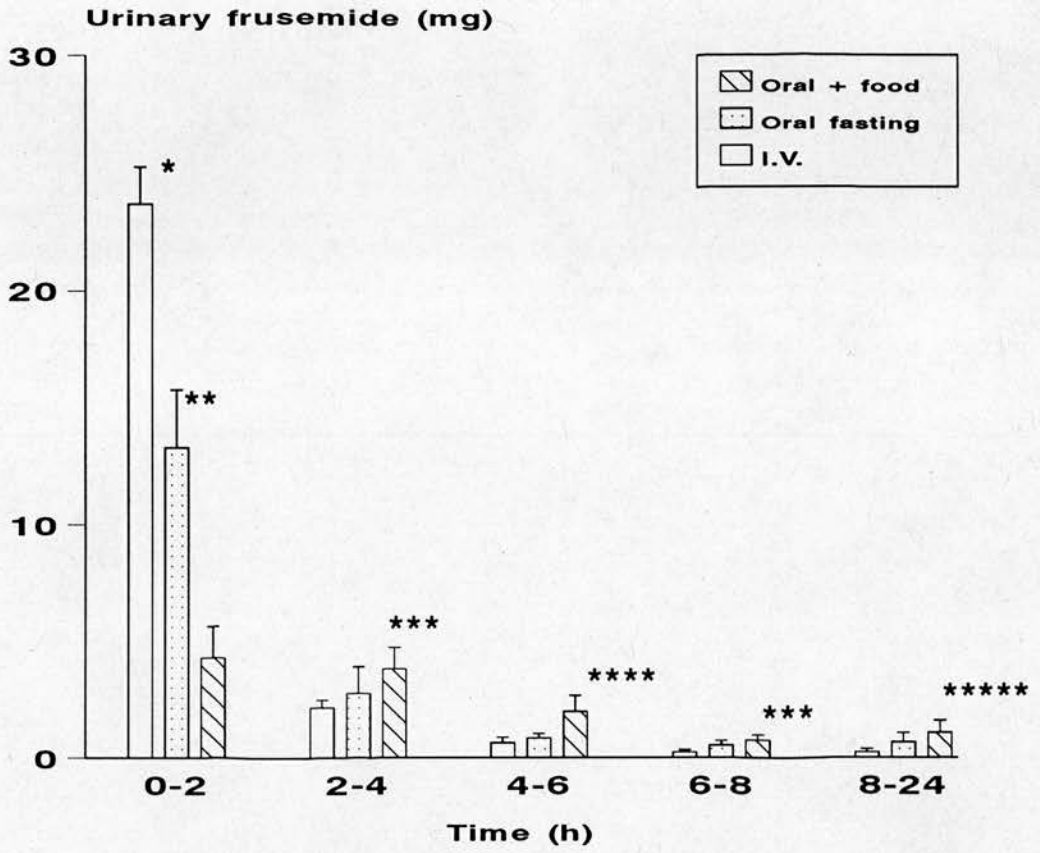


Figure 3.4. Urinary recoveries of frusemide 40mg following intravenous and oral doses with and without food in 8 healthy volunteers. *P<0.001, compared to oral fasting and oral with food: **P<0.001, compared to oral with food: ***P<0.05, compared to i.v. and oral fasting: ****P<0.001, compared to i.v. and oral fasting: *****P<0.01, compared to i.v.

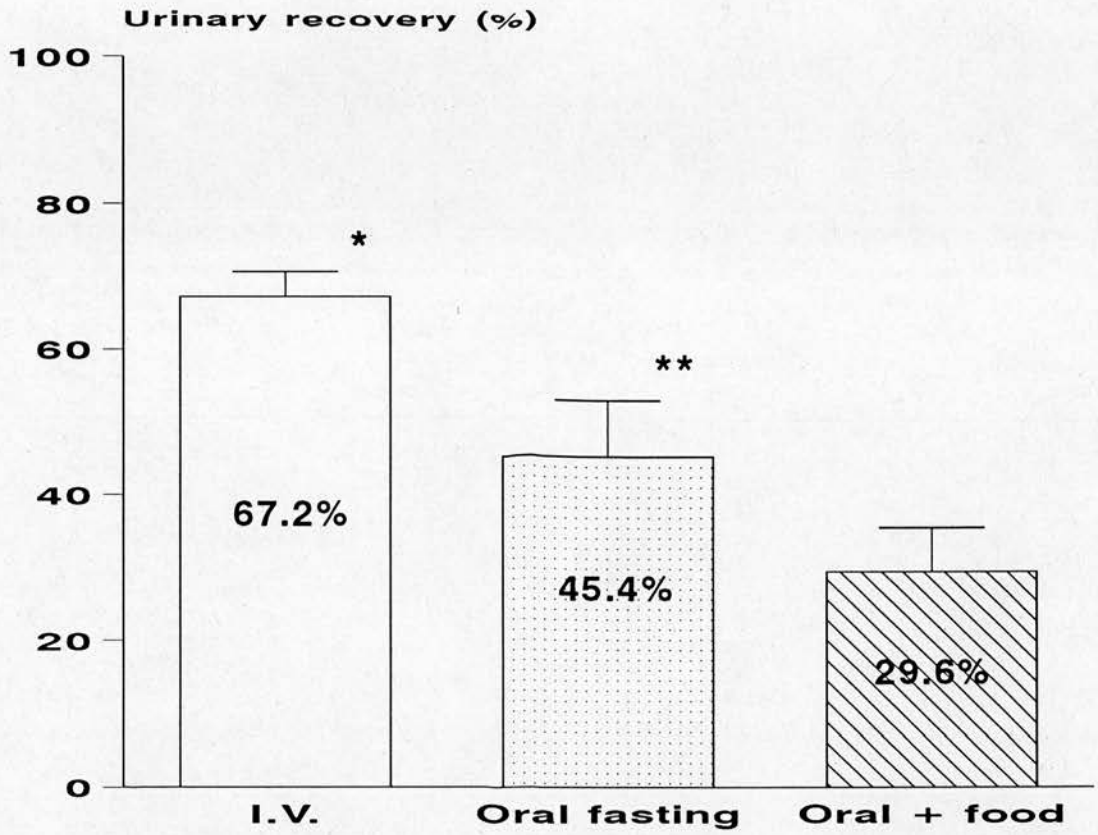


Figure 3.5. Total urinary recovery of frusemide over 24h (% of administered dose) following 40mg intravenous and oral doses with and without food in 8 healthy volunteers. *P<0.001, compared to oral fasting and oral with food: **P<0.001, compared to oral with food.

dose (67.2 ± 3.4 %).

The bioavailability of frusemide was also calculated from urinary recovery data according to Equation 2.11. Using this method the bioavailability of frusemide administered in the fasting state and with food were 67.4 ± 14.4 and 44.2 ± 10.3 %, respectively. These values are not significantly different from the values calculated using the areas under the plasma concentration-time curves.

The renal clearance of frusemide was not significantly lower for any of the oral doses compared to the intravenous dose (See Table 3.2). Food therefore had no obvious influence on the renal clearance of frusemide.

Sodium excretion

The urinary excretion of sodium, potassium and water following the administration of frusemide are only presented for the first 8 h of the study because after this time the volunteers went home and fluid intake was not standardised.

Mean sodium excretion rates and total urinary sodium excretion following intravenous and oral administration of frusemide are given in Figs 3.6 and 3.7. No significant difference in total sodium output over 8 h was found. However differences in patterns of response were observed. Over the first 2 hours sodium excretion was significantly higher for the intravenous dose compared to the oral dose given with food. No significant difference was found between the intravenous and oral fasting dose. Subsequently the rate of sodium excretion declined for both the intravenous and oral fasting dose. From 2 h onwards, sodium excretion rates following the oral

administration of frusemide with food were significantly higher compared with both the intravenous and the oral fasting dose.

Potassium excretion

Mean potassium excretion rates and total potassium output following intravenous and oral administration of frusemide are shown in Figs 3.8 and 3.9. Different patterns of response were again obtained for the three dosage regimes. The intravenous dose produced significantly higher potassium output over the first 2 hours compared to the oral doses. However, over the latter part of the study a delayed response was obtained for the oral dose given with food, producing significantly higher potassium excretion from 2 - 8 h. Total potassium output over 8 h was found to be significantly higher for the intravenous dose and the oral dose given with food compared to the oral fasting dose.

Urine volumes

A similar pattern of response was again observed for mean urinary volumes following intravenous and oral administration of frusemide (Fig 3.10).The intravenous and oral fasting dose produced significantly higher urinary volumes over the first 2 h but from 2-6 h urinary volumes were significantly higher following the oral dose administered with food. Total urine output (See Fig 3.11) for all three doses, over 8 h, was found not to be significantly different.

Side Effects

Subjects 5 and 7 experienced mild transient leg cramp 2 - 3 h after the administration of

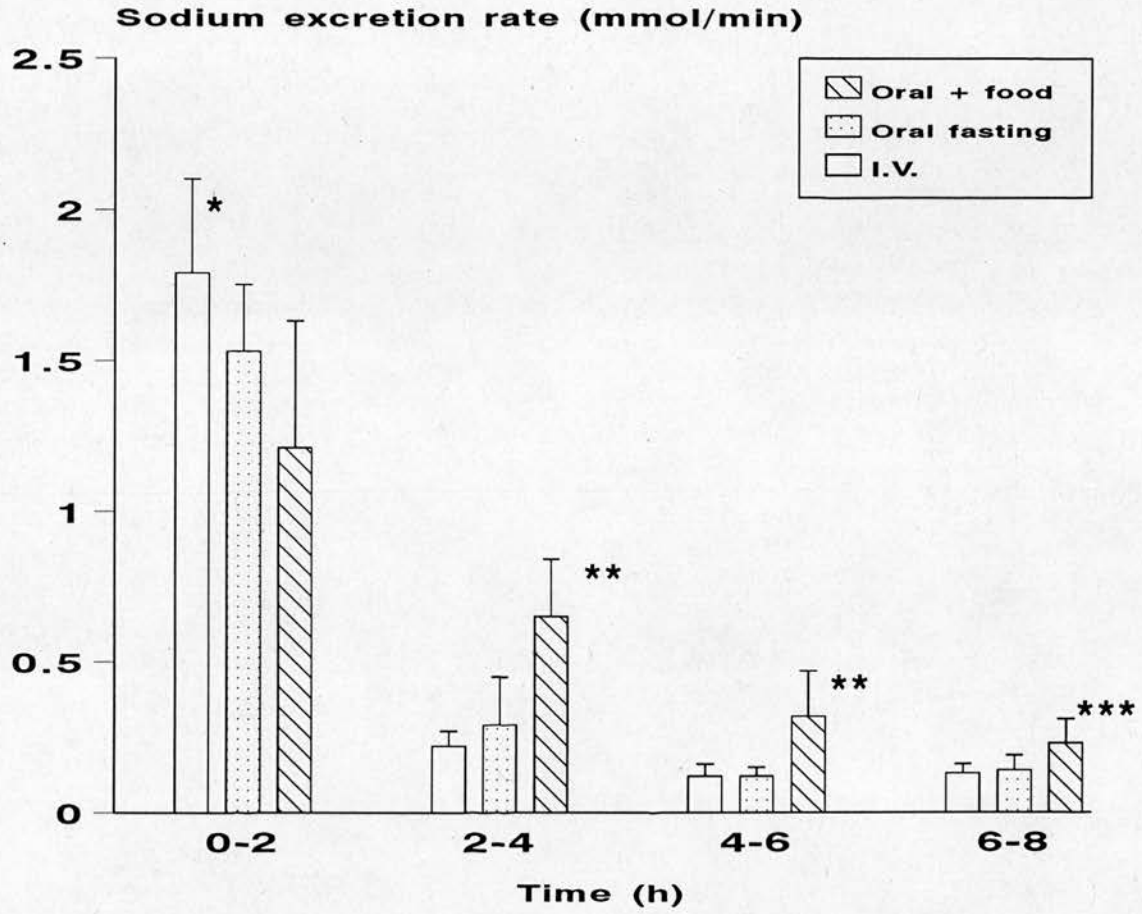


Figure 3.6. Mean sodium excretion rates induced by 40mg intravenous and oral frusemide with and without food in 8 healthy volunteers. *P<0.01, compared to oral with food: **P<0.001, compared to i.v. and oral fasting: ***P<0.05, compared to i.v and oral fasting.

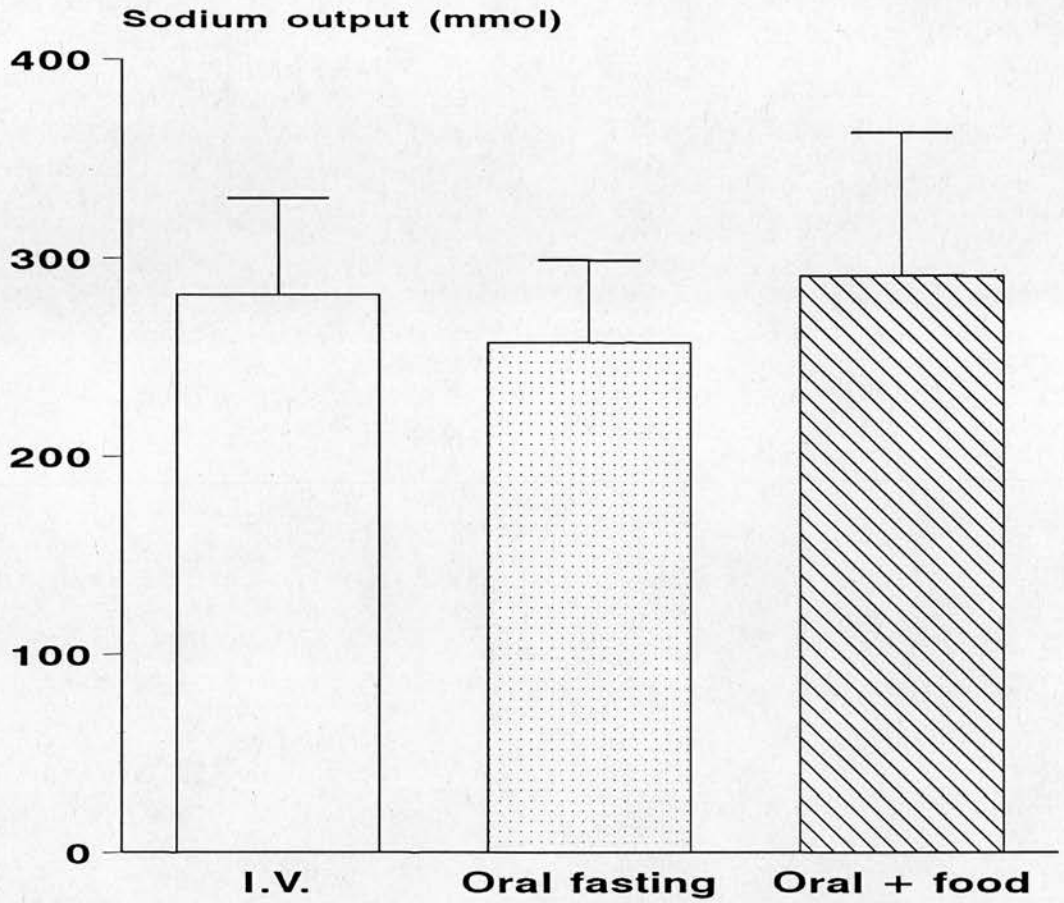


Figure 3.7. Total sodium output over 8h following 40mg intravenous and oral frusemide with and without food in 8 healthy volunteers. No significant differences were found.

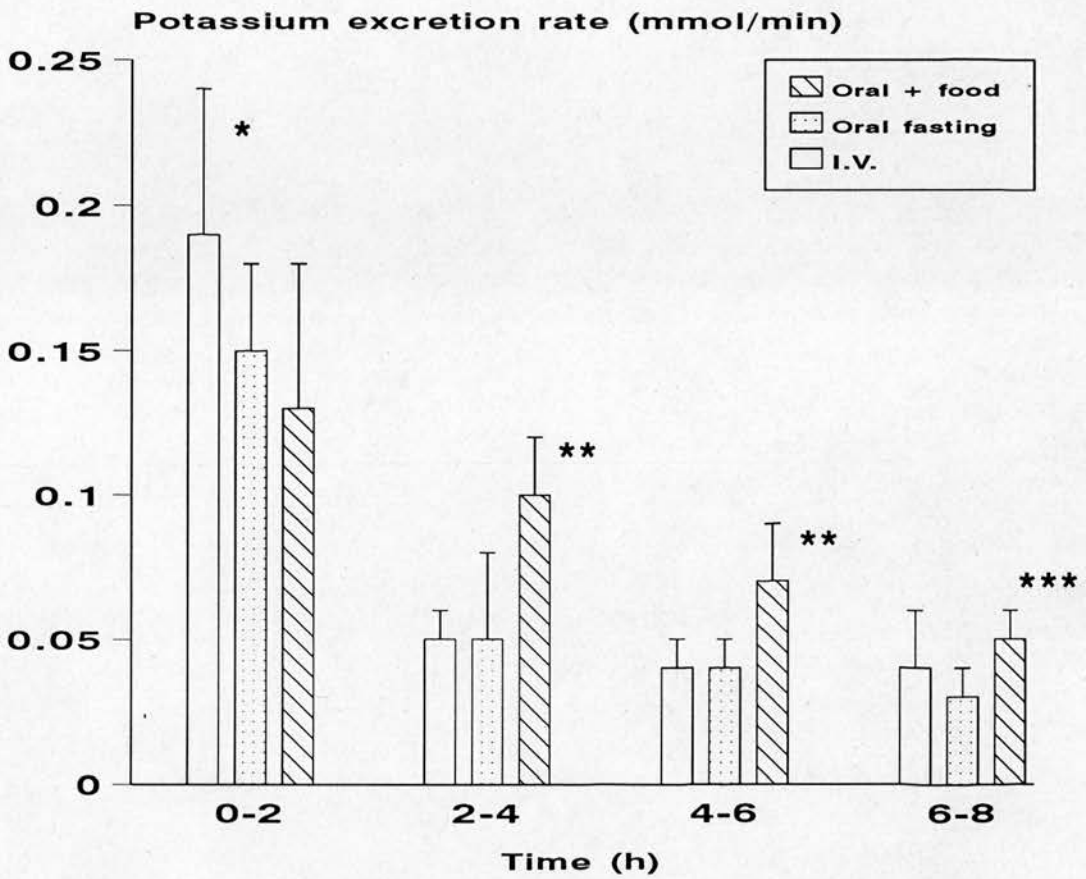


Figure 3.8. Mean potassium excretion rates induced by 40mg intravenous and oral frusemide with and without food in 8 healthy volunteers. *P<0.05, compared to oral fasting and oral with food: **P<0.001, compared to i.v. and oral fasting: ***P<0.05, compared to oral fasting.

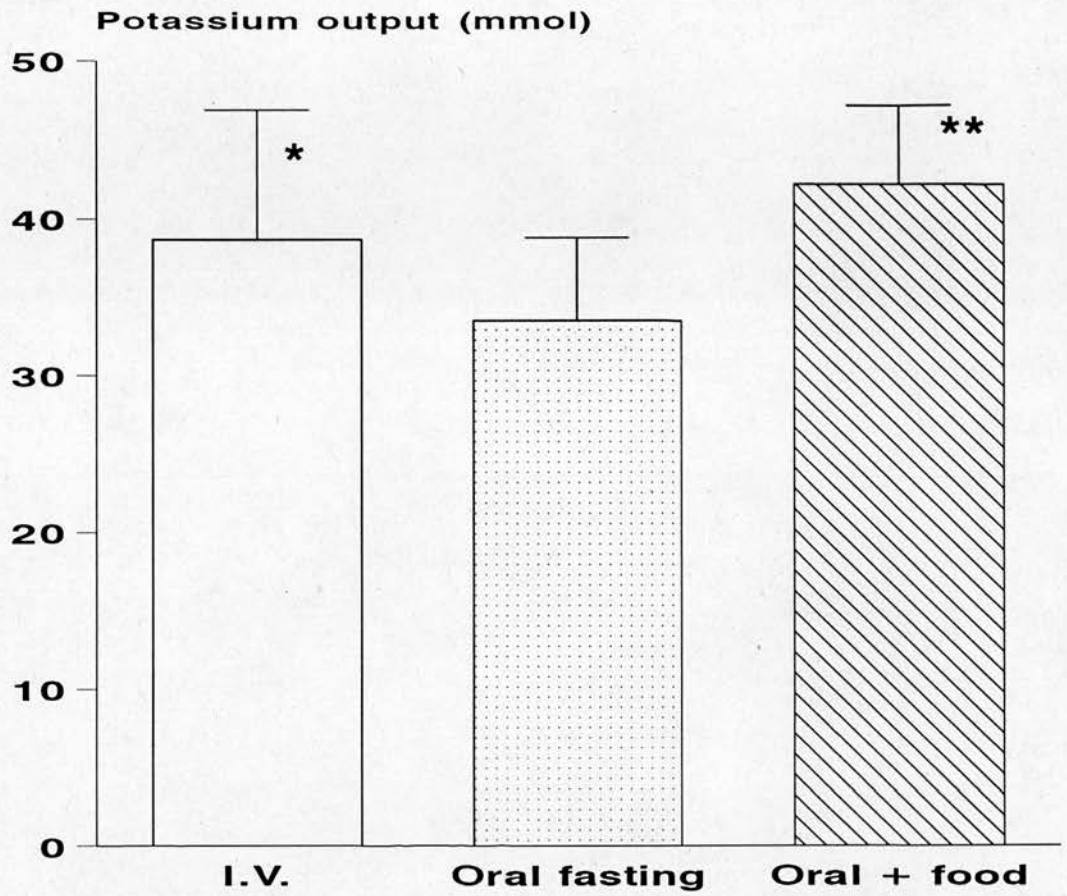


Figure 3.9. Total potassium output over 8h following 40mg intravenous and oral frusemide in 8 healthy volunteers. *P<0.05, compared to oral fasting: **P<0.01, compared to oral fasting. Potassium output was not significantly different after the i.v dose and the oral dose given with food.

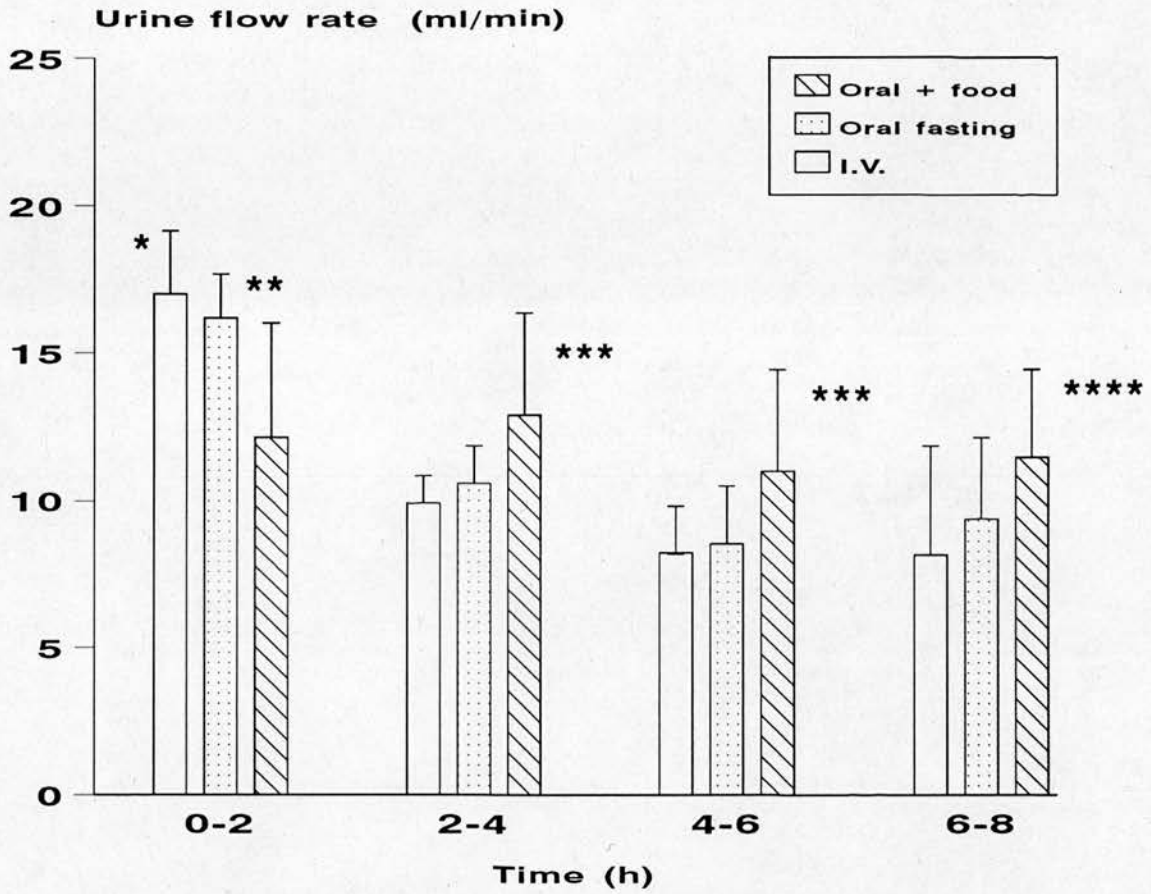


Figure 3.10. Mean urine flow rates induced by 40mg intravenous and oral frusemide with and without food in 8 healthy volunteers. *P<0.01, compared to oral with food: **P<0.05, compared to oral with food: ***P<0.05, compared to i.v. and oral fasting: ****P<0.05, compared to i.v.

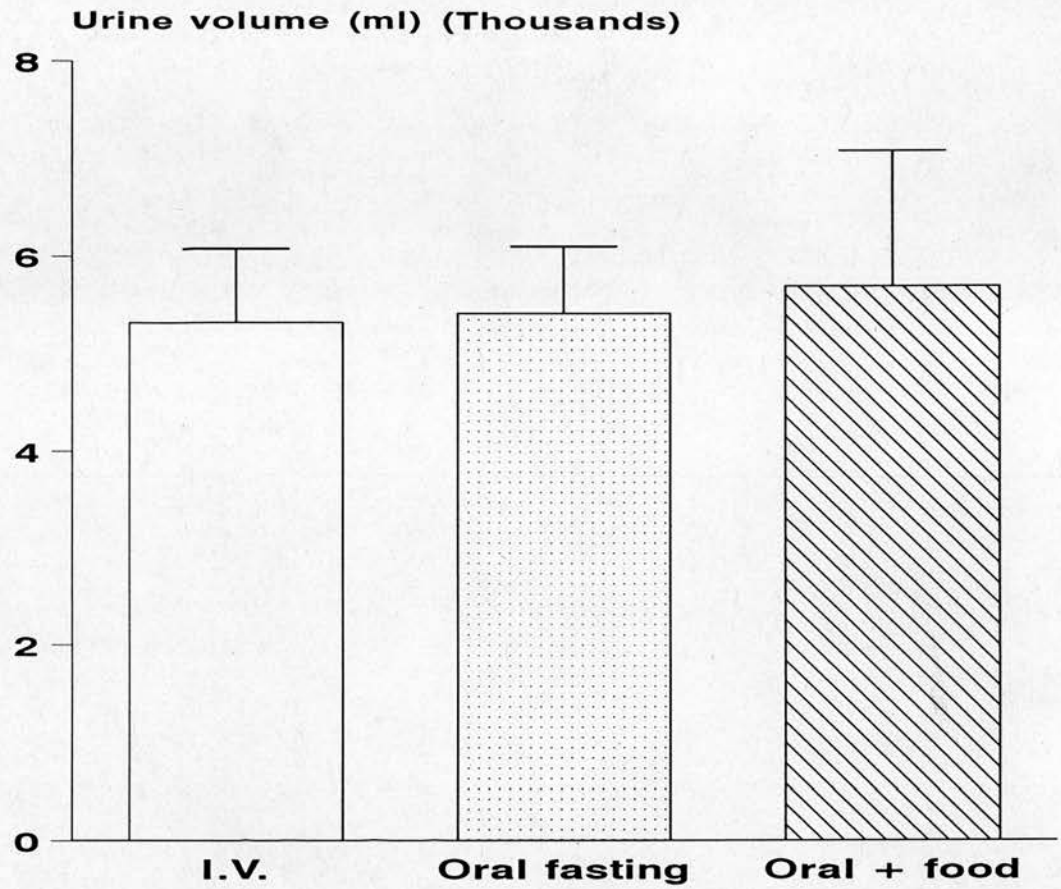


Figure 3.11. Total urine volumes over 8h following 40mg intravenous and oral frusemide in 8 healthy volunteers. No significant differences were found.

the intravenous dose of frusemide. Subject 1 felt slightly light-headed 1 h after administration of the intravenous dose, when standing to pass urine The same subject also complained of a headache after leaving the investigation room, having completed the study. All these symptoms are known side effects of loop diuretics and can be explained by the fluid and electrolyte disturbances caused by frusemide.

Section 3.4. Discussion

Intravenous dose

The pharmacokinetics of frusemide in healthy volunteers have previously been studied by several investigators and are marked by a large degree of variability (Andreasen *et al.* 1982; Chennavasin *et al.* 1981; Cutler and Blair, 1979; Grahnen *et al.* 1984; Kelly *et al.* 1973; Smith *et al.* 1980; Waller *et al.* 1982,1988). These discrepancies in frusemide kinetics occur partly because of the different methods employed to derive the pharmacokinetic values, in addition to differences within and between subjects, study protocols and assay methods used.

After an intravenous dose of frusemide, plasma concentrations have been modelled using 1, 2 and 3 exponential terms (Cutler and Blair, 1979; Hammarlund *et al.* 1984; Kelly *et al.* 1973; Waller *et al.* 1982). Specifically the weighting factor used in fitting these models has been found to greatly influence the final parameter estimates, especially terminal half-life (Chennavasin *et al.* 1981).

In the present study individual data were best fitted using a biexponential equation with weighting factor $1/y^2$. The results, with a mean half-life of 96 mins, total clearance of

147.7 ml/min, renal clearance of 99.1 ml/min and volume of distribution of 0.27 L/kg, are in agreement with previously reported data (See Table3.5).

Oral doses

The extent of oral frusemide absorption in normal subjects appears to be independent of dosage form i.e. solution versus tablet (Kelly *et al.* 1973; Waller *et al.* 1982). Waller *et al* (1982) and Hammarlund *et al* (1984) have found differences in the rate of frusemide absorption between solution and tablet form. They also observed considerable interindividual variation in the shapes of plasma concentration time curves for tablets given in the fasting state. Clinical evidence does seem to support the therapeutic benefit of administration of frusemide as a solution especially in patients with congestive heart failure (Niazov *et al.* 1985).

In the present study frusemide was administered orally as a solution in order to eliminate the added variable of drug dissolution. Using this dosage form very little variation was observed between subjects in the shapes of the plasma concentration time curves following administration in the fasting state.

Food intake however produced a dramatic change in the shapes of the plasma concentration time curves with a significant reduction in Cmax and a delay in Tmax. Absolute bioavailability of frusemide was significantly reduced by approximately 30 %, as judged from both plasma and urine data.

These results contrast with those obtained by Kelly *et al* (1973) and Hammarlund *et al* (1984), both of whom found no significant difference in the bioavailability of frusemide

Table 3.5. Reported frusemide kinetics in healthy volunteers following intravenous administration (mean ± s.d.).

Number of Subjects	Assay	Dose	$t_{1/2}$ b (min)	CL (ml/min)	CL_R (ml/min)	Vd (L/kg)	Reference
7	F + TLC	40	66 ± 29	219 ± 49		0.27 ± 0.06	Andreasen *et al.* 1978
8	F + TLC	40	72 ± 29	166 ± 42	116 ± 67	0.24 ± 0.07	Andreasen & Mikkelson, 1977
5	GLC, HPLC	40	52 ± 15	194 ± 35	95 ± 24	0.21 ± 0.06	Beermann *et al.* 1977
4	HPLC	20,40	100 ± 45	125 ± 73	64 ± 43	0.29 ± 0.06	Chennavasin *et al.* 1979
4	F	40	30 ± 6	160 ± 38	149 ± 37	0.12 ± 0.02	Cutler *et al.* 1974
8	HPLC	80	51 ± 9	158 ± 22	80 ± 22	0.16 ± 0.02	Rane *et al.* 1978
11	^{35}S	22	48 ± 4	113 ± 8		0.09 ± 0.01	Tilstone & Fine, 1978
21	HPLC	40	78	117 ± 41	88 ± 45		Waller *et al.* 1982

Abbreviations: F = Fluorometric; TLC = Thin layer chromatography; CLG = Gas liquid chromatography; HPLC = High performance liquid chromatography.

given with and without food.

The study by Kelly *et al* (1973) was however terminated 4 h after frusemide administration thereby making calculations of $AUC_{0-\infty}$ inaccurate, especially for the dose administered after food. The assay used was also much less sensitive and there were highly variable results in the 24 h frusemide excretion.

Hammarlund *et al* (1984) , using a very similar breakfast to Beermann and Midskov (1986), did find a reduction in AUC compared to the fasting dose, although the difference did not achieve significance. On the other hand, Hammarlund *et al* (1984) did find a significant fall in the urinary recovery of frusemide. Beermann and Midskov (1986) suggested that the lack of a significant difference in AUC when frusemide was administered with and without food may have been a Type 2 error.

The significant reduction in the bioavailability of frusemide solution given with food in this study however, strengthens the findings of Beermann and Midskov (1986) who also found that a meal decreased the bioavailability of a frusemide tablet by 30 %. Taken together these results suggest that the reduction in bioavailability is not related to dissolution problems of frusemide in the presence of food. A number of other factors may however be responsible:

(1) Prolongation of gastric emptying time

A predominant effect of food is inhibition of stomach emptying, due, primarily to feedback mechanisms situated in the small intestine (Toothaker and Welling, 1980; Ponto and Schoenwald, 1990). However Beermann and Midskov (1986) also found an

almost identical reduction in frusemide bioavailability when the drug was given with a heavy meal indicating that the effect was not caused by stomach emptying.

(2) Physical interaction

Absorption or adsorption interaction between frusemide and food components may influence drug bioavailability.

(3) Metabolism

There is evidence to suggest that frusemide is metabolised in the stomach (Lee and Chiou, 1983). If frusemide remains in the stomach for a longer period, as when administered with food, this metabolism may be increased thereby reducing the amount of intact frusemide available for absorption.

Response to frusemide

In spite of the pronounced differences found in the extent of absorption and excretion of frusemide between the intravenous and oral doses, no significant difference in total sodium and water excretion was found. Some caution must be taken when comparing these results since basal sodium excretion rates were not determined.

However, this type of discrepancy between the total amount of frusemide excreted and the total effect has been observed previously by several other investigators. Branch *et al* (1977) and Kelly *et al* (1973) found equivalent responses for 80 mg frusemide administered orally and intravenously despite the lower bioavailability of frusemide or less frusemide being excreted after the oral dose. Kaojarern *et al* (1982), after obtaining

a similar response, proposed that the time course of frusemide delivery to its luminal site of action may influence the total response independent of the total amount of drug excreted. They calculated a maximally efficient excretion rate for frusemide and found that oral administration maintains the drug close to this amount more persistently than the intravenous dose.

Another possible explanation of the results may be that the more sustained urinary levels of frusemide found after administration with food, may interrupt a period of relative sodium retention (i.e. acute tolerance). The development of acute tolerance with frusemide is believed to be due to activation of renal compensatory mechanisms (Hammarlund *et al.* 1985; Sjostrom *et al.* 1988b). Hammarlund *et al* (1985) suggested that direct isovolumetric substitution of urine losses produces little or no tolerance. However Li *et al* (1986), using the dog as a model, showed that no fluid replacement or 100 % replacement with 5 % dextrose in water after intravenous frusemide both produced the same degree of acute tolerance in natriuresis indicating the insignificance of water compensation in tolerance development.

Overall, the results from this study not only underline the importance of carrying out drug bioavailability studies in fasting and non-fasting states but also the importance of determining whether any alteration in drug kinetics produces a change in drug response.

Having said this, these results were obtained in healthy volunteers receiving a single dose of frusemide immediately after a standard breakfast with replacement of urine losses. They are therefore still not easily applicable clinically where patients usually receive frusemide long term and may ingest it together with food in a more random manner.

CHAPTER 4

EFFECTS OF FOOD ON THE BIOAVAILABILITY OF BUMETANIDE

Section 4.1. Introduction

Bumetanide is a natriuretic and diuretic agent belonging to the same class of "loop diuretics", as frusemide. Bumetanide is however 40-60 times more potent than frusemide on a weight basis (Asbury *et al.* 1972; Brater *et al.* 1983a; Davies *et al.* 1974; Murdoch & Auld, 1975; Ramsay *et al.* 1978) and has a greater bioavailability of approximately 80 % versus 50 % (Brater *et al.* 1983a; Holazo *et al.* 1984; Cook *et al.* 1988).

The previous study has shown that the absorption of frusemide in healthy volunteers is grossly impaired when administered with a standard breakfast. Little evidence exists as to whether the absorption of bumetanide is similarly affected by the presence of food. Homeida *et al* (1976) did find a delayed response to oral bumetanide when given to healthy volunteers after a meal, but no pharmacokinetic data were reported.

The aim of this study was to determine whether the bioavailability of bumetanide was altered when administered with food.

Section 4.2. Methods

Subjects

Nine healthy male volunteers aged 19 to 36 years (27 ± 5 yr) and weighing 53 to 79 kg (67 ± 7 kg) took part in the study. Subjects 1, 2 and 6 had participated in the previous frusemide study. All volunteers were healthy according to medical history, clinical examination and haematological and biochemical tests. They were negative for HIV and

Hepatitis B antigen. All volunteers were informed of the nature of the study and each gave written informed consent. The study was approved by the Lothian Health Board Healthy Volunteers Studies of Ethics of Medical Research Sub-Committee. The volunteers were asked to:

(1) avoid alcohol for 24 h before each study day
(2) avoid taking any other medication for 1 week prior to and throughout the study
(3) fast from 22.00 h the night before each study day

Procedure

The nine volunteers received bumetanide 2 mg solution (0.5 mg/ml "Burinex" injection) intravenously and orally with and without breakfast exactly as described for frusemide in Chapter 3, Section 3.2.

Venous blood samples were taken just before dosing and at 5, 10, 15, 30, 45, 60, 90, 120, 150, 180 min and hourly for the next 5 h after intravenous administration. After an oral dose samples were taken at 0, 10, 15, 30, 45, 60, 90, 120, 150, 180 min and hourly for the next 5 h. All samples were collected in lithium heparin tubes and centrifuged at 3000 rpm for 15 minutes. Plasma was stored at -20^{0}C.

Urine was collected half-hourly for the first 3 h and then hourly up to 8 h for both intravenous and oral studies. Volunteers also completed an 8-24 h urine collection at home. Urine volumes were recorded and aliquots stored at -20^{0}C until analysis. Bumetanide was shown to be stable under these condition for at least 12 weeks (See Chapter 2).

Analysis of samples

Concentrations of bumetanide in plasma and urine were measured by high performance liquid chromatography with fluorescence detection as described in Chapter 2. Urinary sodium and potassium concentrations were analysed by ion specific electrodes.

Pharmacokinetic analysis

Plasma concentration time data were analysed using the "Siphar" curve fitting and modelling program.

Plasma concentration time curves following intravenous administration were best described by a two compartment model comprising distribution and elimination phases according to Equation 2.14. Following oral bumetanide administration in the fasting state plasma concentration time curves were fitted to a one compartment model with 2 phases according to Equation 2.12. Plasma concentration time profiles for oral administration with food could not be fitted to the model because of irregular absorption. The trapezoidal rule was used to calculate $AUC_{0\text{-}8\ h}$ for these data and the area beyond the last measured concentration (C_t) to infinity was estimated according to:

$$AUC_{t\text{-}\infty} = C_t / k_{el}$$

where k_{el} = intravenous elimination rate constant.

Statistics

All data are expressed as mean ± standard deviation (s.d.) unless otherwise stated.

Analysis of variance was used to determine statistical differences between doses and $P<0.05$ was accepted as significant.

Section 4.3. Results

Intravenous administration

The mean plasma concentration time plot for bumetanide following intravenous administration is shown in Fig 4.1. Bumetanide concentrations could only be measured up to 5 h in all individuals. The mean elimination half life was 67 ± 11 min (Table 4.1).

The observed total plasma clearance (CL) for bumetanide was 202.8 ± 52.3 ml/min (Table 4.2). Renal clearance (CL_R) contributed 71.4 ± 8.4 % to the total body clearance of bumetanide. Non renal clearance (CL_{NR}) was 58.6 ± 22.2 ml/min. The mean volume of distribution was 19.03 ± 3.95 L or 0.28 ± 0.07 L/kg.

The mean area under the curve after intravenous bumetanide was 175.4 ± 45.0 μg.h/l. The total amount of unchanged bumetanide excreted in the urine over 24 h was 1.43 ± 0.17 mg or 71.4 ± 8.5 % of the administered dose. Although urine was collected up to 24 h, bumetanide could not be detected after 8 h, except in one subject.

Oral administration

Mean plasma concentration time curves for oral doses given with and without food are shown in Fig 4.2. Individual plasma concentration time curves are shown in Fig 4.3. Food altered the shapes of the curves by reducing Cmax and prolonging Tmax, in most individuals. Mean values for Cmax, Tmax and $AUC_{0-\infty}$ are given in Table 4.3. The mean

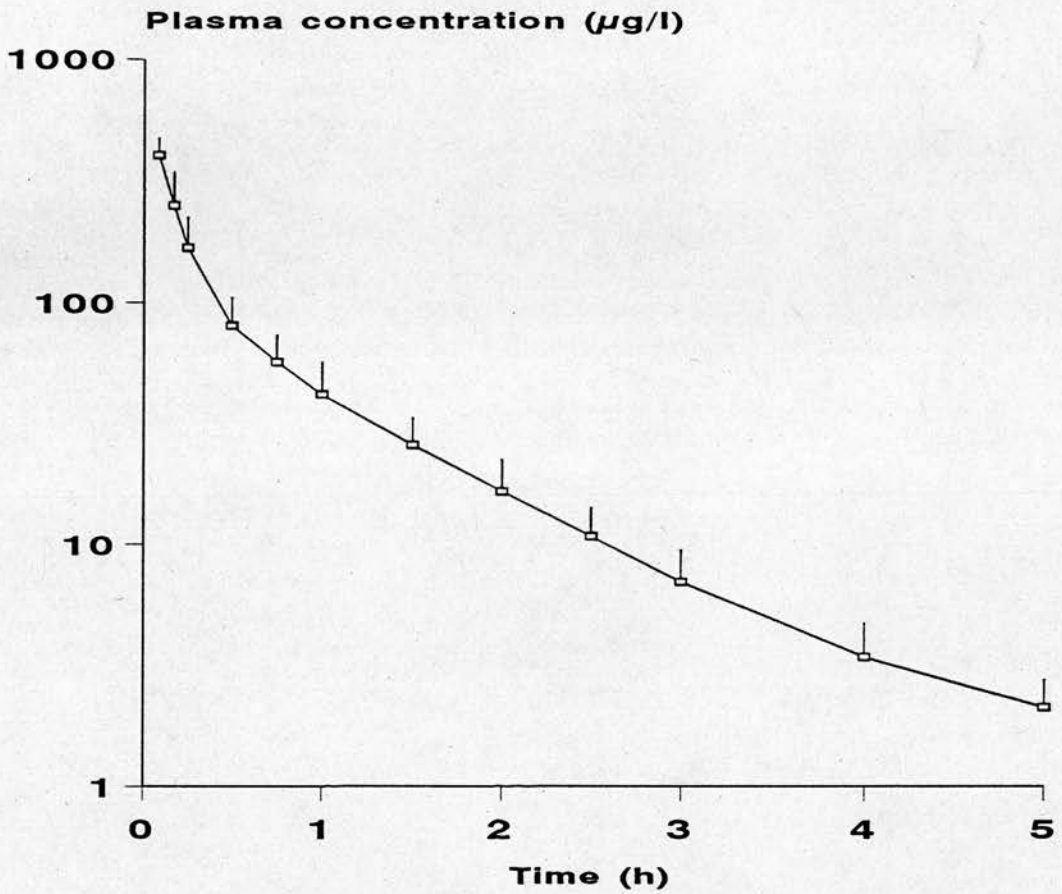


Figure 4.1. Mean plasma concentrations following intravenous administration of 2 mg bumetanide over 5 minutes to 9 healthy volunteers. Bars = ± s.d.

Table 4.1. Pharmacokinetic data of bumetanide 2 mg given intravenously (mean ± s.d.).

Intercept/ Constant	Parameter estimate ± s.d.	Half-life (min)
D(μg/l)	410±100	10±5
K_d (h^{-1})	5.55 ± 3.11	
B(μg/l)	50 ± 10	67±11
K_{el} (h^{-1})	0.64 ± 0.11	

Table 4.2. Total plasma clearance (CL), renal clearance (CL_r) and volume of distribution (Vd) for bumetanide after intravenous and oral administration of 2 mg.

Subject	CL (ml/min)	CL_r (ml/min)			Vd_{area}
	iv. dose	iv dose	oral fasting	oral with food	(L/kg) (iv. dose)
1	195.3	130.8	120.3	65.0	0.36
2	279.7	187.3	157.3	190.0	0.34
3	214.3	165.0	138.3	55.5	0.40
4	193.2	111.2	113.3	153.3	0.26
5	293.7	218.8	211.5	175.0	0.34
6	210.0	148.0	142.3	183.7	0.21
7	160.3	116.2	122.0	151.7	0.19
8	127.2	84.5	114.5	117.0	0.23
9	151.8	136.7	168.3	140.0	0.24
Mean	202.8	144.3	143.1	136.8	0.28
±s.d.	52.3	38.8	30.2	46.1	0.07

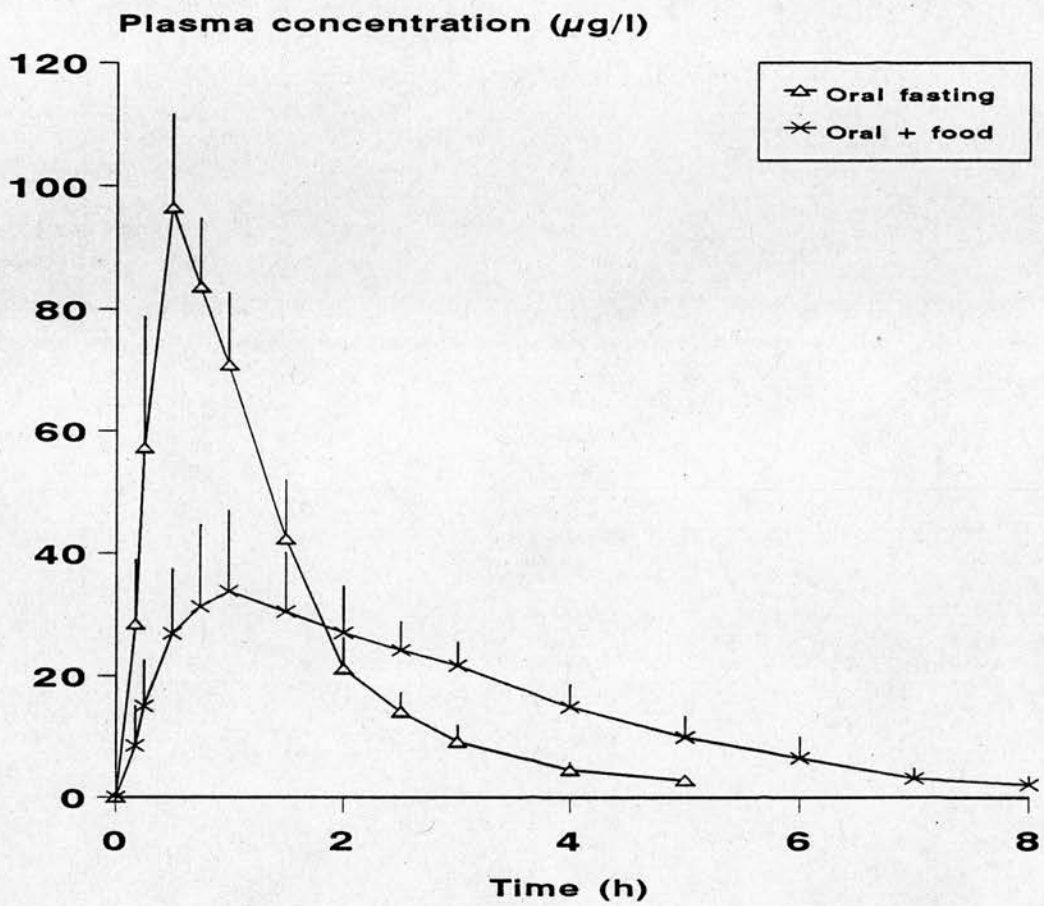


Figure 4.2. Mean plasma concentrations following oral administration of 2 mg bumetanide with and without food to 9 healthy volunteers. Bars = ± s.d.

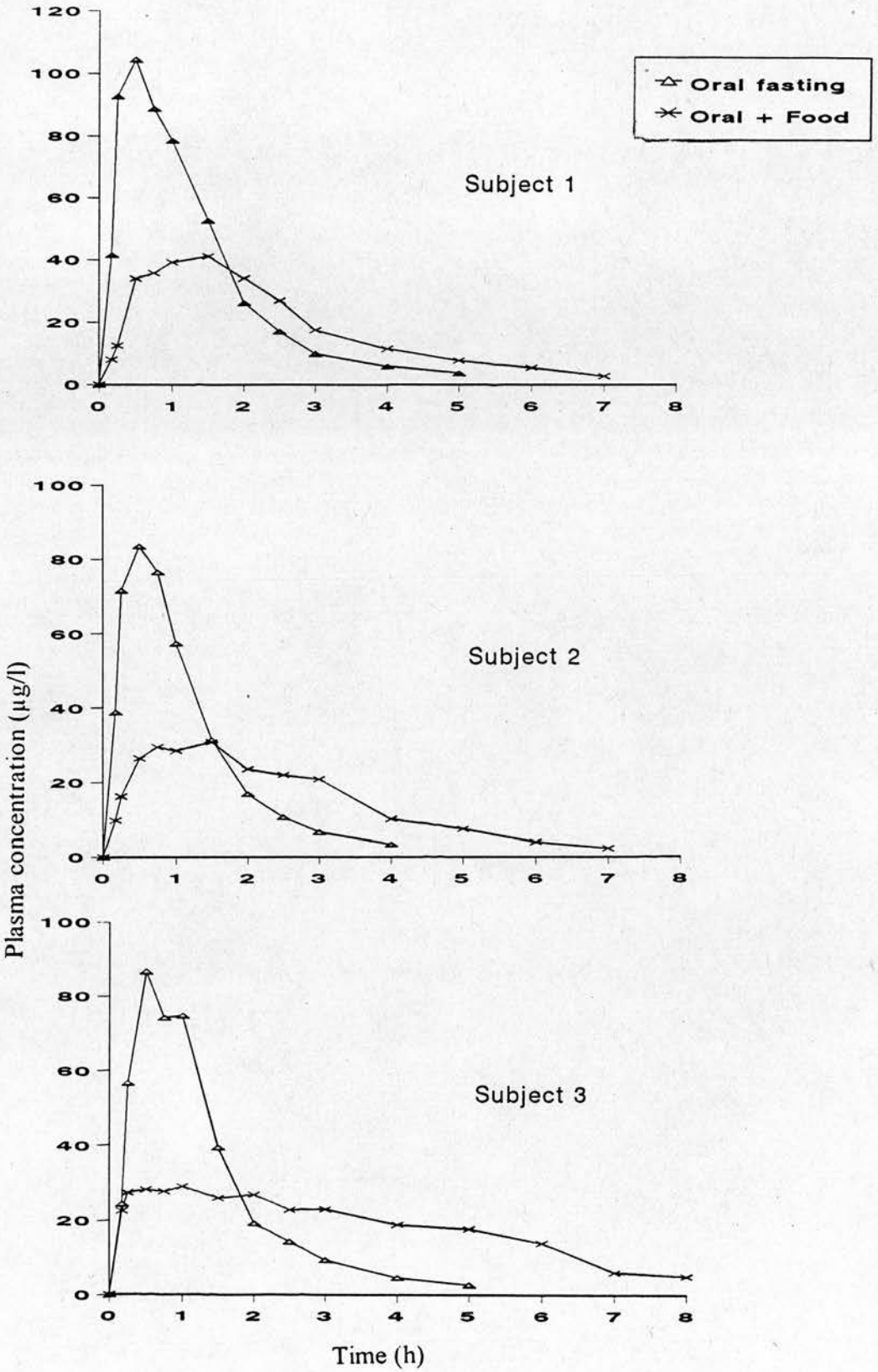


Figure 4.3. Individual plasma concentrations following oral administration of 2 mg bumetanide with and without food.

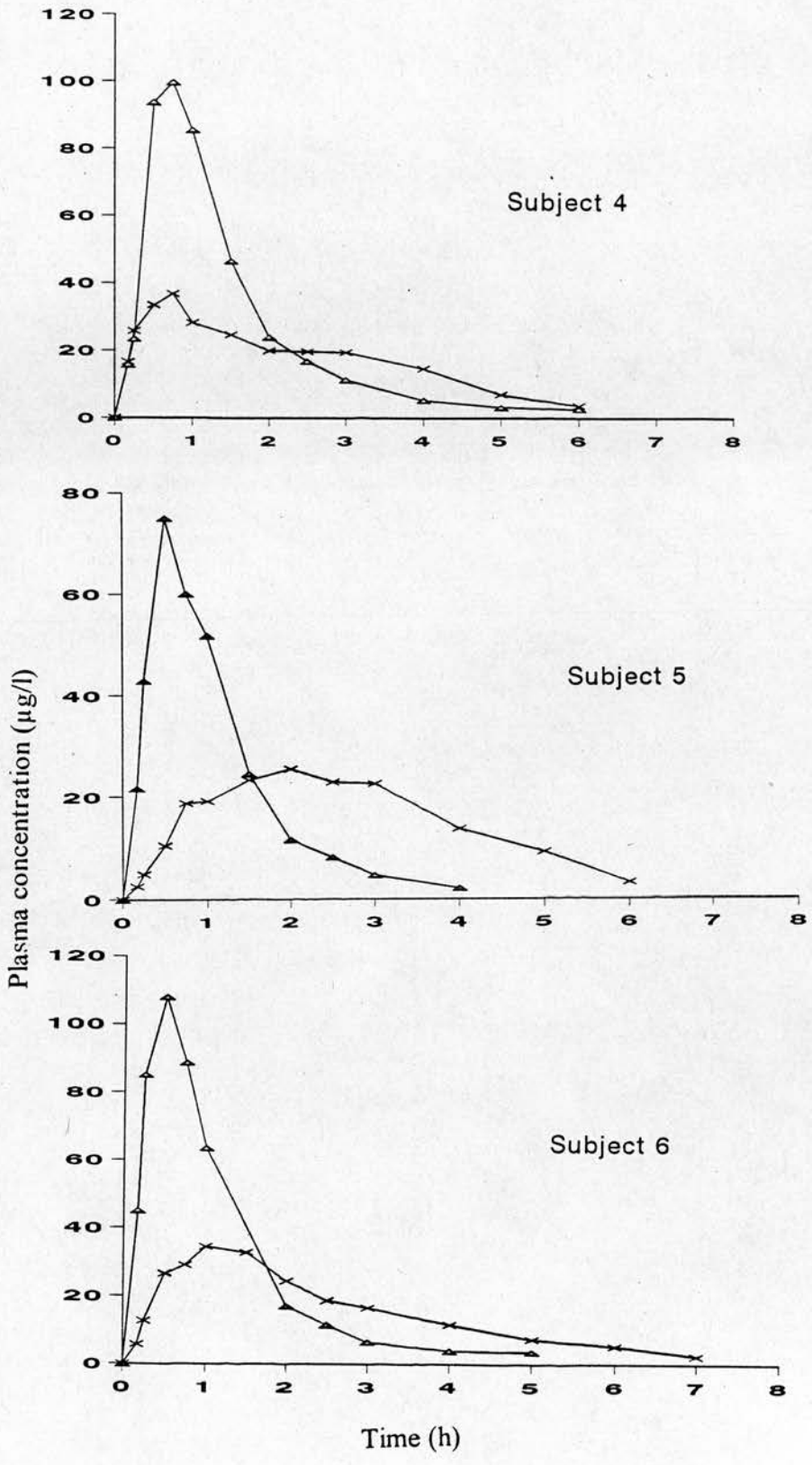


Figure 4.3. Continued.

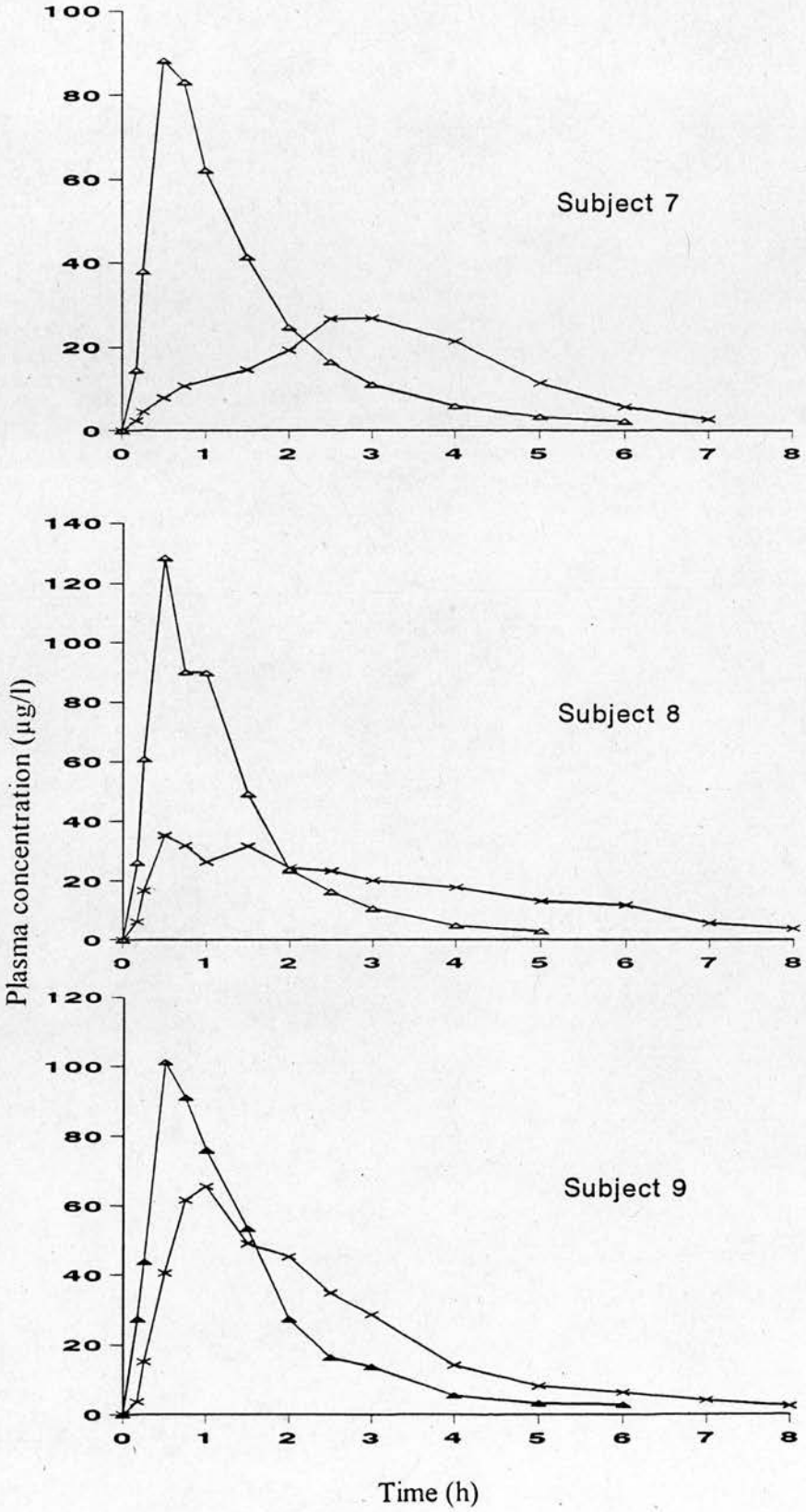


Figure 4.3. Continued.

Table 4.3. Peak concentrations (Cmax), time to peak concentrations (Tmax) and area under the plasma concentration time curves ($AUC_{0-\infty}$) following oral administration of 2 mg bumetanide to 9 healthy volunteers with and without food.

Subject	Cmax (µg/l)		Tmax (h)		$AUC_{0-\infty}$ (µg.h/l)	
	Without food	With food	Without food	With food	Without food	With food
1	104.5	41.1	0.50	1.50	164.8	130.6
2	83.4	30.7	0.50	1.50	116.5	112.3
3	86.7	28.9	0.50	1.00	133.7	150.2
4	96.6	36.8	0.75	0.75	164.7	111.9
5	74.9	25.4	0.50	2.00	96.1	97.2
6	107.9	34.4	0.50	1.00	142.9	110.7
7	88.3	26.8	0.50	3.00	140.8	105.7
8	128.2	35.3	0.50	0.50	167.4	141.0
9	101.2	65.6	0.50	1.00	162.3	179.2
Mean	96.9	36.1	0.53	1.36	143.2	126.5
± s.d.	15.1	11.5	0.08	0.72	23.0	24.8
P	<0.001		<0.05		NS	

peak plasma concentration of 96.9 ± 15.1 μg/l occurred 0.53 ± 0.08 h after oral administration of bumetanide in the fasting state. Cmax was significantly reduced to 36.1 ± 11.5 μg/l ($P<0.001$) and Tmax delayed to 1.36 ± 0.72 h ($P<0.05$) following administration with food. However no significant difference was found in mean $AUC_{0-\infty}$ values for the oral doses given with (126.5 ± 24.8 μg.h/l) and without (143.2 ± 23.0 μg.h/l) food.

Bioavailability

The bioavailability of oral bumetanide was calculated using Equation 2.10. Results are given in Table 4.4. The bioavailabilities of bumetanide given with and without food were 74.8 ± 15.5 and 83.7 ± 12.4 %, respectively. This difference was not statistically significant, although there was a tendency towards lower values after food intake.

Renal elimination

Urinary recoveries of bumetanide following oral and intravenous administration are shown in Fig 4.4. Over the first 2 h, urinary recovery was significantly higher for the intravenous dose compared to the oral doses given with and without food. Recovery was also significantly higher for the oral fasting dose compared to the dose administered with food, over the same period. However from 2-8 h, recovery of bumetanide was significantly higher following oral administration with food compared to the intravenous and oral fasting doses. Urinary excretion of oral bumetanide was virtually complete 8 h after drug administration. From 8-24 h urinary bumetanide could only be detected in 3 subjects following the oral fasting dose. This represented 1.7 ± 1.1 % of the total amount

Table 4.4. Bioavailability of 2 mg oral bumetanide administered with and without food to 9 healthy volunteers.

Subject	$AUC_{0-\infty}$ (µg.h/l) iv.	Bioavailability (%) Without food	With food
1	170.7	96.5	76.5
2	119.2	97.7	94.2
3	155.5	86.0	96.6
4	172.5	95.5	64.9
5	113.5	84.7	85.6
6	158.1	90.4	70.0
7	208.0	64.9	50.8
8	262.1	63.9	53.8
9	219.5	73.9	81.6
Mean	175.4	83.7	74.8
±s.d.	45.0	12.4	15.5
P		NS	

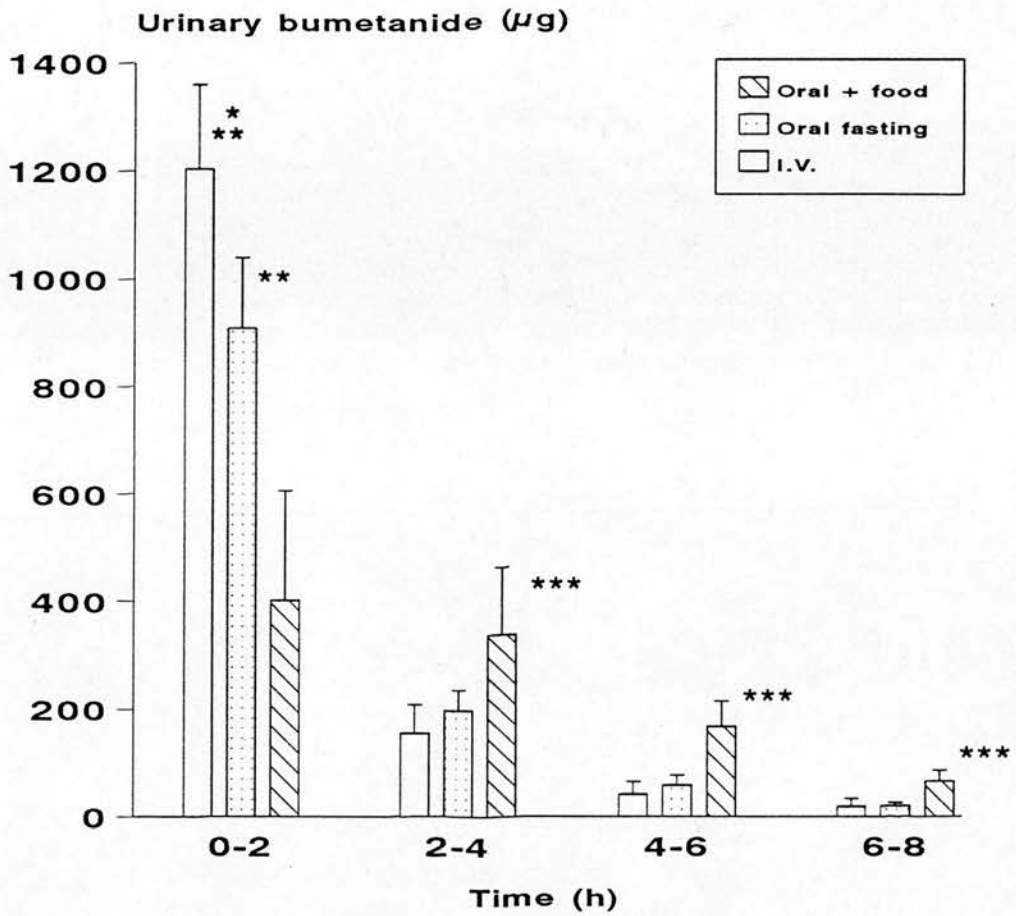


Figure 4.4 Urinary recoveries of bumetanide following 2mg intravenous and oral doses with and without food in 9 healthy volunteers. *P<0.01, compared to oral fasting: **P<0.001, compared to oral with food: ***P<0.001, compared to i.v. and oral fasting.

of drug excreted unchanged. After the oral dose given with food, bumetanide could be detected in 6 out of the 9 subjects and represented 2.6 ± 1.8 % of the total bumetanide recovered.

Mean total urinary recovery of bumetanide during 24 h (Fig. 4.5) following oral administration with and without food averaged 50.1 ± 15.6 and 59.7 ± 8.4 %, respectively. The difference was not statistically significant. Both recoveries were lower than with the intravenous dose (71.4 ± 8.5 %).

The bioavailability of bumetanide was also calculated from the urinary recovery using Equation 2.11. Bioavailability was 84.3 ± 8.3 % when administered in the fasting state and 70.5 ± 22.2 % when administered with food. These values are not significantly different from those calculated using $AUC_{0-\infty}$.

No significant differences were found in the renal clearance of bumetanide for the oral doses given with and without food and the intravenous dose (Table 4.2).

Sodium excretion

Urinary excretion of sodium, potassium and water following administration of bumetanide are only reported for the first 8 h of the study. All volunteers then went home and fluid intake was not standardised.

Mean sodium excretion rates and total urinary sodium output following intravenous and oral administration of bumetanide are shown in Figs. 4.6 and 4.7.

A difference in the pattern of response was obtained between the 3 dosage regimes.

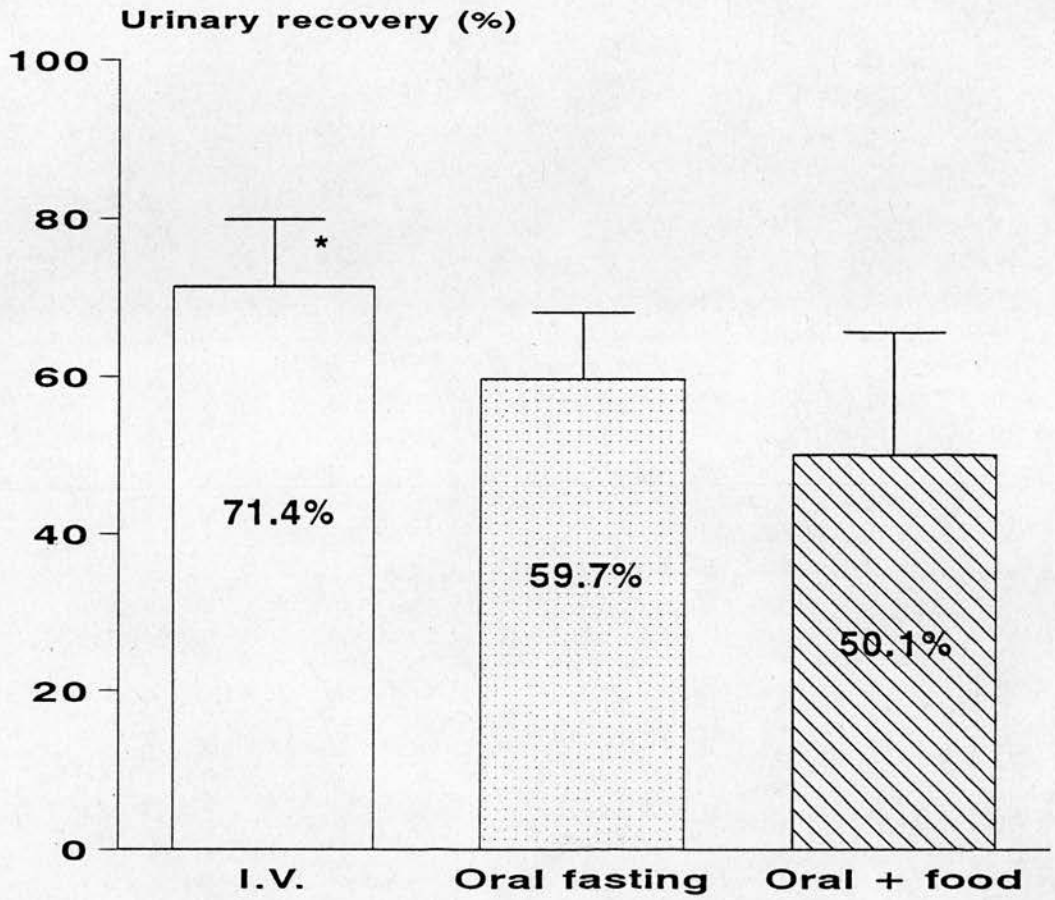


Figure 4.5. Total urinary recovery of bumetanide over 24h (% of administered dose) following 2mg intravenous and oral doses with and without food in 9 healthy volunteers. *P<0.01, compared to oral fasting and oral with food.

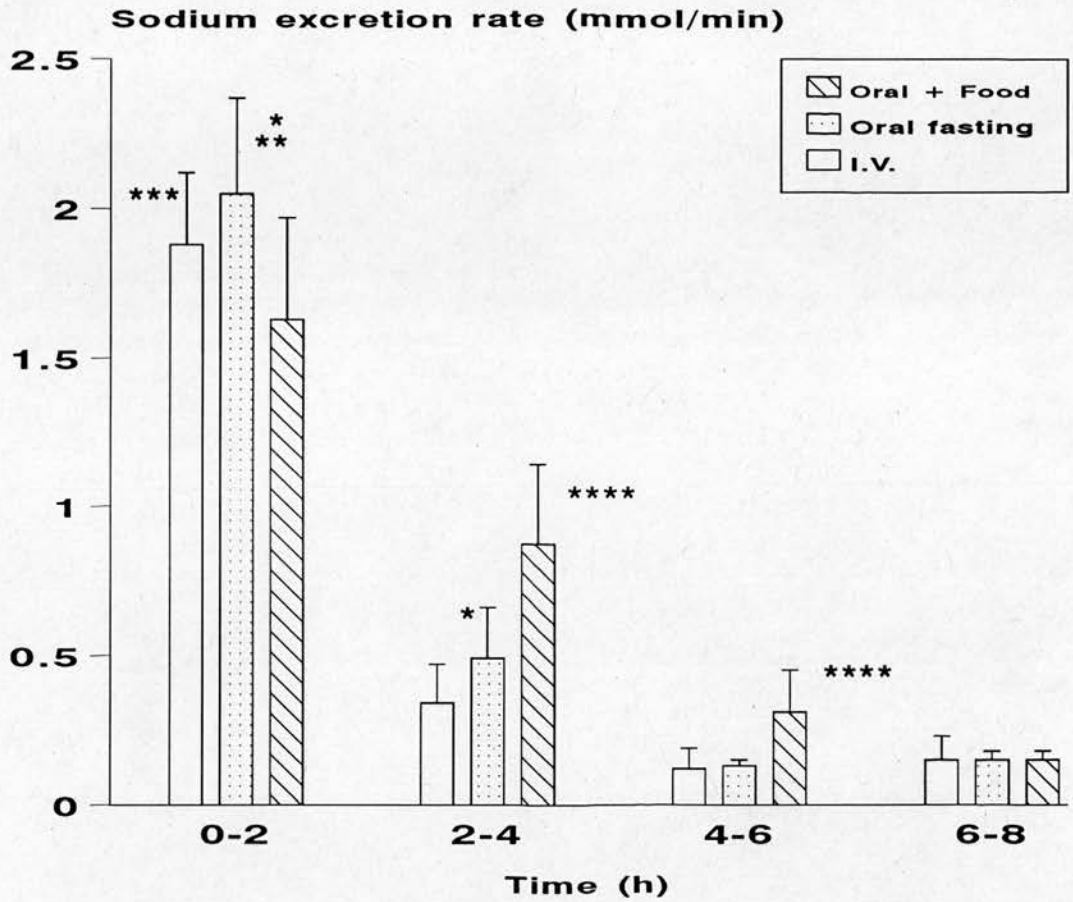


Figure 4.6. Mean sodium excretion rates induced by 2mg intravenous and oral bumetanide with and without food in 9 healthy volunteers. *P<0.05, compared to i.v.: **P<0.001, compared to oral with food: ***P<0.01, compared to oral with food: ****P<0.001, compared to i.v. and oral fasting.

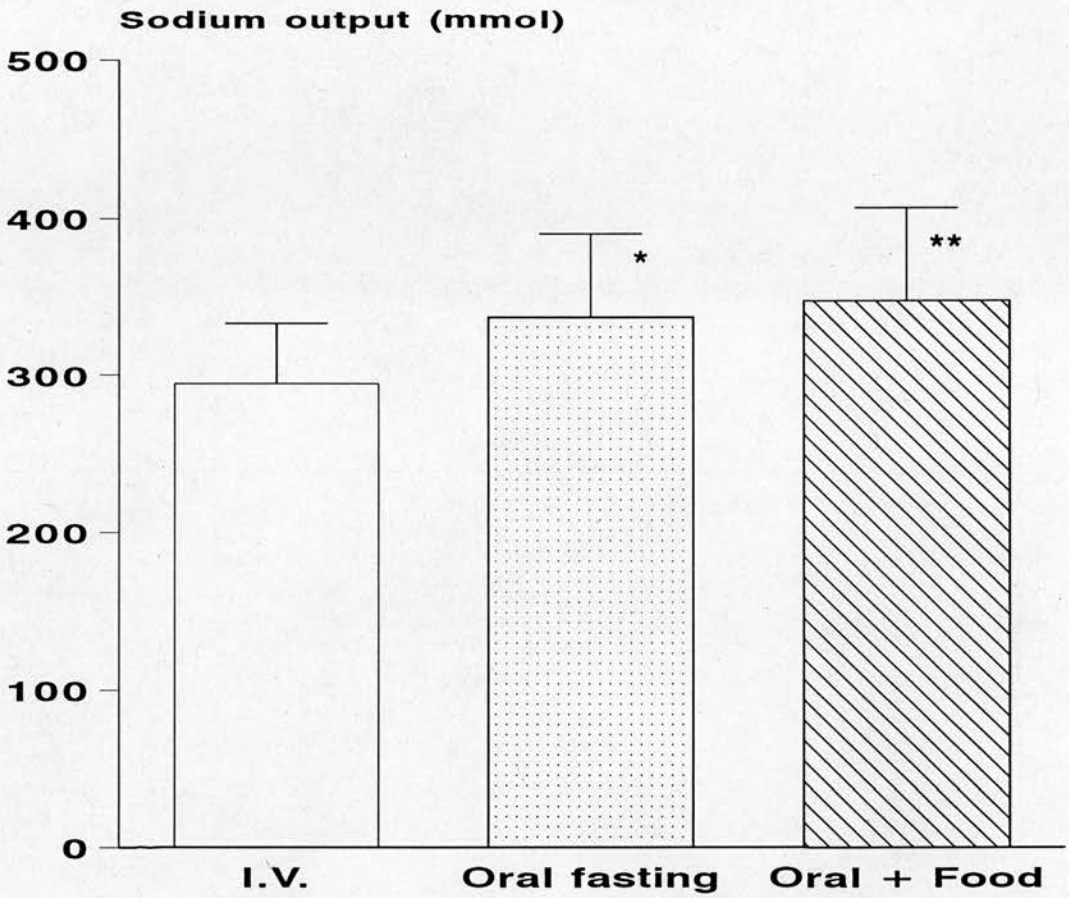


Figure 4.7. Total sodium output over 8h following 2mg intravenous and oral bumetanide with and without food in 9 healthy volunteers. *P<0.01, compared to i.v.: **P<0.001, compared to i.v. No significant difference was found between the oral fasting and oral dose given with food.

Over the first 2 h sodium excretion rate was significantly higher for the oral fasting dose compared to the intravenous dose and the dose administered with food. In the same period, sodium excretion rate was also significantly higher for the intravenous dose compared to the oral dose given with food. However from 2-6 h sodium excretion rates following oral administration with food were significantly higher compared to both the intravenous and oral fasting doses.

Over 8 h, total sodium output was found to be significantly higher for both oral doses compared to the intravenous dose.

Urine flow rate

A similar pattern of response was obtained for urine flow rates following intravenous and oral bumetanide (Fig. 4.8). The oral dose produced a significantly higher urine flow rate over the first 2 h compared to the intravenous dose and the oral dose given with food. From 2-4 h urine flow rate was higher for both the oral doses compared to the intravenous dose and from 4-6 h it remained significantly higher for the oral dose given with food.

Total urine volume over 8 h (Fig. 4.9) was significantly higher following the oral doses of bumetanide given with and without food compared to the intravenous dose.

Potassium excretion

Mean urinary potassium excretion rates following intravenous and oral bumetanide administration are shown in Fig. 4.10. During the first 2 h no significant difference in

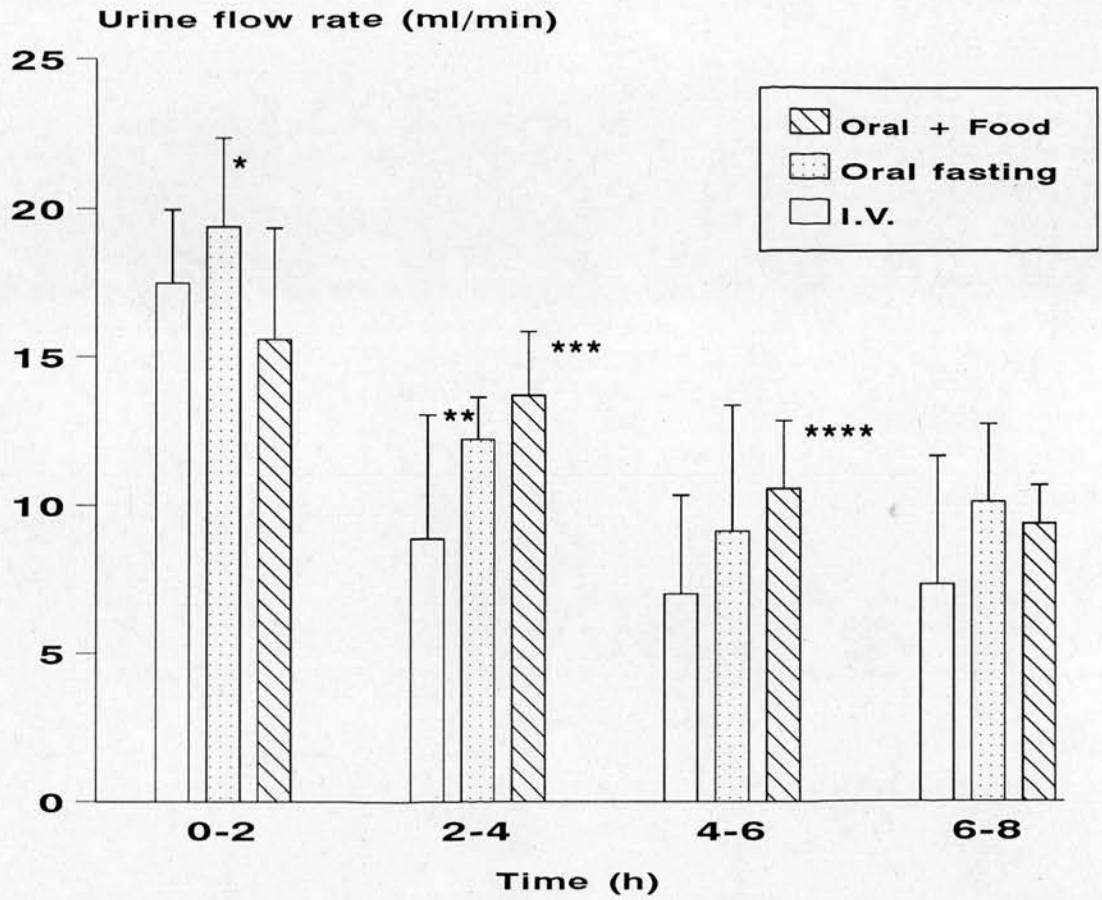


Figure 4.8. Mean urine flow rates induced by 2mg intravenous and oral bumetanide with and without food in 9 healthy volunteers. *P<0.01, compared to oral with food: **P<0.05, compared to i.v.: ***P<0.01, compared to i.v.: ****P<0.05, compared to i.v.

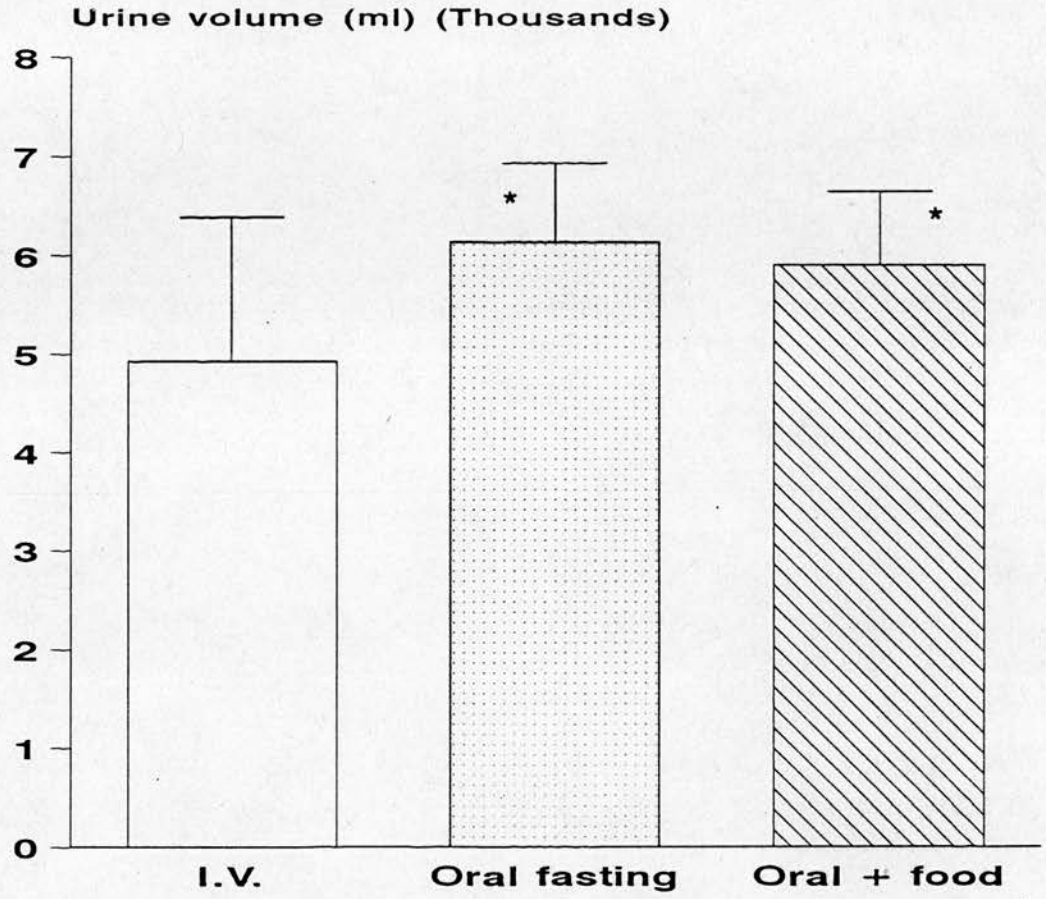


Figure 4.9. Total urine volumes over 8h following 2mg intravenous and oral bumetanide with and without food in 9 healthy volunteers. *P<0.01, compared to i.v. Urine volume was not significantly different after the oral fasting dose and the oral dose given with food.

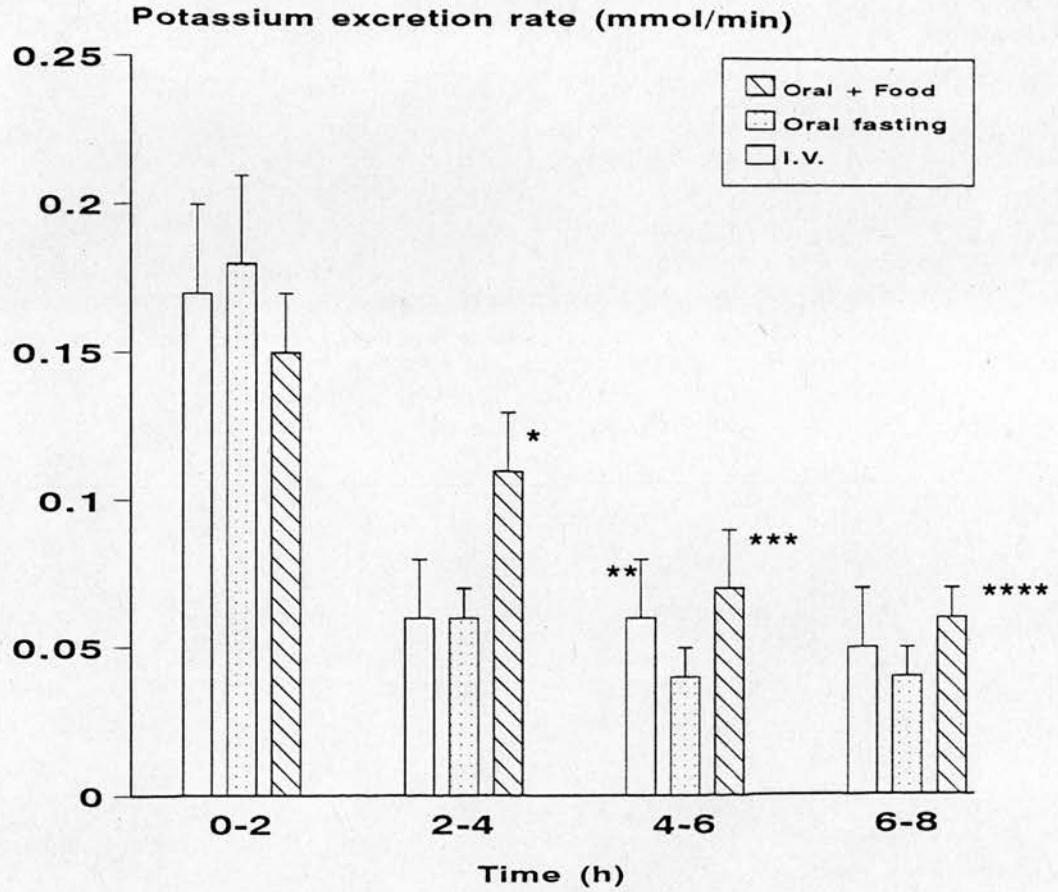


Figure 4.10. Mean potassium excretion rates induced by 2mg intravenous and oral bumetanide with and without food in 9 healthy volunteers. *P<0.001, compared to i.v. and oral fasting: **P<0.05, compared to oral fasting: ***P<0.01, compared to oral fasting: ****P<0.05, compared to oral fasting.

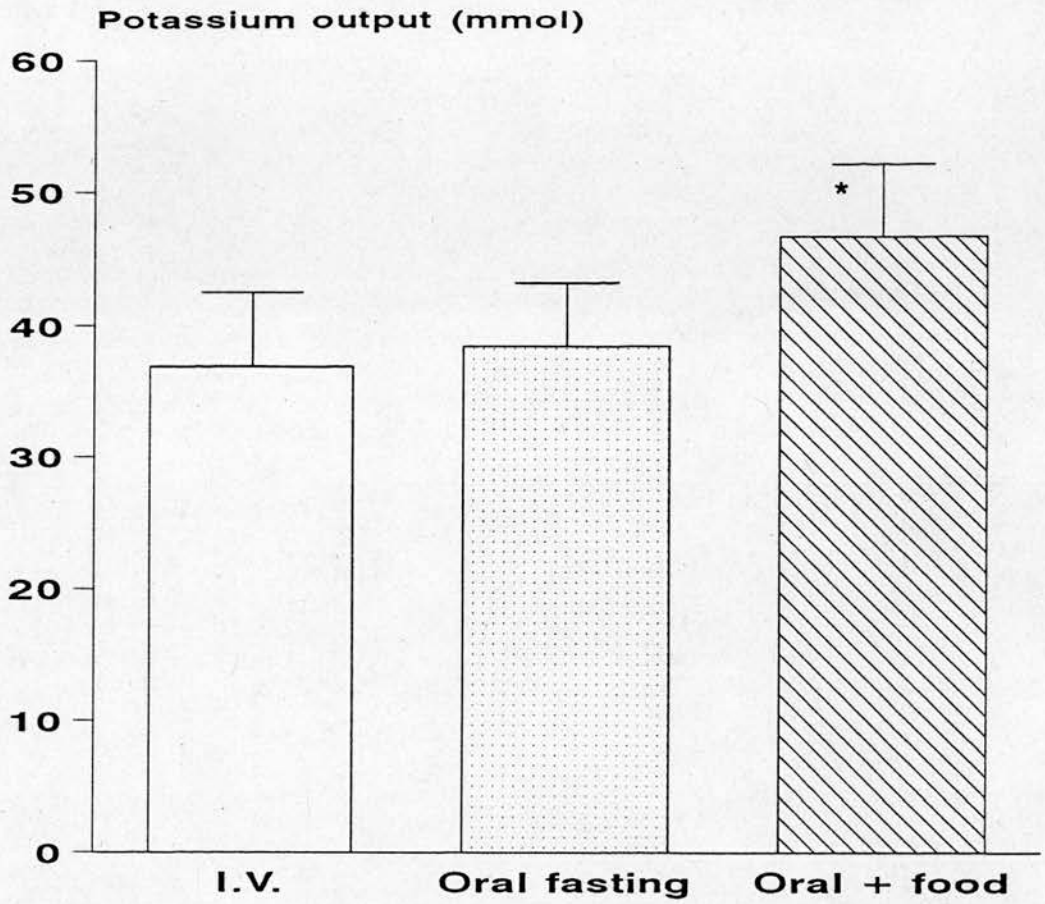


Figure 4.11. Total potassium output over 8 h following 2 mg intravenous and oral bumetanide with and without food in 9 healthy volunteers. *P<0.01, compared to i.v. and oral fasting. No significant difference in potassium output was found between the i.v. and oral fasting doses.

potassium excretion was found. From 2-4 h potassium excretion rate was higher for the oral dose given with food compared to the intravenous and oral fasting doses and from 4-8 h it remained higher compared to the oral fasting dose.

Total potassium excretion over 8 h (Fig. 4.11) was significantly higher for the oral dose given with food compared to the oral fasting and the intravenous dose.

Section 4.4. Discussion

Intravenous administration

A variety of pharmacokinetic models have been used to describe the disposition and elimination of bumetanide in healthy volunteers (Table 4.5). Halladay *et al* (1977) described the elimination of bumetanide using a one compartment model, while Davies *et al* (1974) used a two compartment open model. Pentikainen *et al* (1980), who was able to measure ^{14}C-bumetanide in plasma for up to 10 h, reported that plasma concentration time data were best described in terms of a three compartment model. However because the percent of drug eliminated during the last exponential phase is small (17-20%), a two compartment model is most often utilised.

In the present study a biexponential equation was used to describe the pharmacokinetics of bumetanide following intravenous infusion. Using this model the terminal elimination half life was 67 ± 11 min. This was of the same magnitude as that reported by others who used either one or two compartment models (Table 4.5). Bumetanide half life was found to be approximately three time longer (181 min) by Pentikainen *et al* (1980) who adopted the three compartment model. However they stated that the half life of the

Table 4.5. Comparison of reported bumetanide pharmacokinetic data obtained in normal subjects (mean ± SEM)

No. of Subjects	Assay	Dose	Model	$t_{1/2}\beta$ (min)	CL (ml/min)	CL_R (ml/min)	CL_{NR} (ml/min)	Vd (L/kg)	Reference
2	GLC	2mg iv	2 comp.		202 ± 85	101 ± 5	101 ± 2	0.27 ± 0.06	Davies *et al*, 1974
8	RIA	2 mg o		72	255 ± 26	107 ± 11	148 ± 19	0.24 ± 0.07	Dixon *et al*, 1976
4	^{14}C	2mg o	1 comp.	80					Halladay *et al*, 1977
12	RIA	1mg iv	2 comp.	44	208 ± 65	146 ± 59	62 ± 24	0.21 ± 0.06	Holazo *et al*, 1984
8	HPLC	1mg iv	3 comp.	202	119 ± 25	77 ± 14	42 ± 15	0.29 ± 0.06	Marcantonio *et al*, 1982
8	HPLC	1mg iv	2 comp.	64	129 ± 10	83 ± 6	46 ± 6	0.12 ± 0.02	Marcantonio *et al*, 1982
4	^{14}C	0.5 mg iv	3 comp.	181	228 ± 15	113 ± 21	115 ± 7	0.16 ± 0.02	Pentikainen *et al*, 1980

second phase, 46 min, may be considered as the true elimination $t_{1/2}$ since most of the drug was eliminated during that phase.

Following intravenous bumetanide, renal elimination was found to be the major elimination pathway. Renal clearance contributed 71 % to the overall elimination of bumetanide. By contrast, some other studies (Table 4.5) have shown equal and sometimes greater elimination of bumetanide by non-renal pathways (Davies *et al.* 1974; Dixon *et al.* 1976; Pentikainen *et al.* 1980). Evidence that hepatic metabolism and biliary excretion contribute to the clearance of bumetanide has been reported in previous studies with ^{14}C-labelled bumetanide (Pentikainen *et al.* 1977). After intravenous administration, metabolites of bumetanide accounted for approximately 30-35 % of total radioactivity excreted into urine (Pentikainen *et al.* 1977), whereas 10-20 % of an oral dose was recovered in the faeces, mostly in the form of alcohols (Halladay *et al.* 1975; Pentikainen *et al.* 1977).

The systemic clearance of bumetanide (202.8 ± 52.3 ml/min) was somewhat greater compared to that found for frusemide in the previous study (147.7 ± 28.3 ml/min, Table 3.2), leading to the shorter half life of bumetanide (67 min versus 96 min for frusemide). The volume of distribution found for intravenous frusemide and bumetanide were similar, 0.27 ± 0.10 and 0.28 ± 0.07 L/kg, respectively. The high degree of plasma protein binding found for both drugs restricts the apparent volume of distribution (Johnson & Johnson, 1990; Lau *et al.* 1986; Ponto & Schoenwald, 1990).

Oral administration

The extent of bioavailability of bumetanide from oral tablet and solution dosage forms are equivalent (Holazo *et al.* 1984). Bumetanide was administered orally as a solution in this study in order to make it comparable with the previous study involving frusemide.

Food significantly reduced the peak concentration of bumetanide and delayed the time to the peak concentration, thereby changing the shape of the plasma concentration time curve. The mean bioavailability of oral bumetanide given in the fasting state, calculated from both plasma and urine data, was 84 %. This was slightly, but not significantly decreased by food. The urinary recovery of bumetanide given with and without food were also found not to be significantly different. Compared with frusemide, the bumetanide solution seems therefore to be affected to a lesser extent by the presence of food. One explanation may be differences in the sites of drug metabolism. As mentioned earlier, there is some evidence to suggest that frusemide metabolism may occur in the stomach (Lee & Chiou, 1983). This metabolism may be increased when the drug is administered with food since it remains in the stomach for a longer time. Bumetanide metabolism is however known to be hepatic in origin (Pentikainen *et al.* 1977, 1985; Schwartz, 1981).

Response to bumetanide

Although more bumetanide was excreted unchanged in the urine over 24 h following intravenous administration, the total amount of water and sodium excreted was significantly higher after oral administration. Even in the first 2 h of the study, mean

urine flow rate and sodium excretion rate were significantly higher for the oral fasting dose compared to the intravenous dose, despite approximately 24 % less drug reaching the urine. This difference may be attributable to dissimilar prestudy conditions because baseline sodium excretion rates were not measured before each treatment. However, similar results have been reported by other workers. Marcantonio *et al* (1982) found greater total water, sodium, and potassium excretion in 24 h after oral administration of 1 mg bumetanide compared to an equivalent intravenous dose. Holazo *et al* (1984) demonstrated comparable cumulative diuretic activity following 1 mg intravenous, intramuscular and oral bumetanide administration to normal subjects. Bumetanide has also been found to elicit equivalent natriuresis and urine flow in patients with congestive heart failure, whether given orally or intravenously and despite 20 % less bumetanide reaching the urine after oral administration (Cook *et al.* 1988).

Similar observations were also found for frusemide in the previous study, with equivalent response being found for oral and intravenous administration, again despite less drug being excreted after the oral doses. Kaojarern *et al* (1982) calculated the existence of a maximally efficient excretion rate for frusemide. As a result, if the drug can remain close to this rate for a prolonged period during oral dosing, the same or greater cumulative response can occur with less total drug reaching the urine. This may also explain the larger response to oral bumetanide found in this study.

These results have shown the bioavailability of an oral bumetanide solution is affected to a lesser extent by the presence of food compared with frusemide. Conclusions about the absorption of a certain drug cannot therefore be derived from studies with other, albeit

related, compounds. However, a general assumption that the absorption of bumetanide in unaffected by food cannot be made since this study involved one preparation of the drug given with and without a more or less standard breakfast. The impact of different types of meals should also be tested.

CHAPTER 5

TIME OF ADMINISTRATION OF FRUSEMIDE AND BUMETANIDE IN RELATION TO FOOD IN HOSPITAL PATIENTS

Section 5.1. Introduction

Frusemide and bumetanide are both commonly used in the treatment of congestive heart failure and oedema associated with hepatic and renal disease (Ponto and Schoenwald, 1990; Ward and Heel, 1984). It has been shown that the absorption of frusemide is greatly reduced when administered to healthy volunteers with food. Bumetanide absorption seemed to be affected to a lesser extent.

The aim of this study was therefore to obtain information on the general use of frusemide and bumetanide in two medical wards within the Edinburgh Royal Infirmary by recording doses, routes of administration and in particular times of administration in relation to meals. If, as seemed likely, the drugs were frequently given with meals, their efficacy could be compromised.

Section 5.2. Methods

The study was approved by the Lothian Health Board Medicine and Clinical Oncology Research Ethics Sub-Committee. The consultant physician in charge of the patients in wards 23 and 24 of the Edinburgh Royal Infirmary were asked if their patients could participate in the survey.

A questionnaire was used to collect patient information, a copy of which is given in Appendix 1. Information was collected over a 3 month period and all evaluable patients admitted to wards 23 and 24, who were receiving frusemide or bumetanide, were studied once 24 -72 h after admission.

The diagnoses and reasons for diuretic treatment were obtained from the patients medical notes. The ward provided information on the other drugs the patient were receiving together with doses, routes of administration and any dose changes in frusemide or bumetanide since admission. After the early morning ward drug round, the patients themselves were asked when they received their frusemide or bumetanide in relation to breakfast. They were also asked at what time they usually took their diuretic at home in relation to breakfast.

Section 5.3. Results and Discussion

The results of the survey are listed in Appendix 2 and summarised below. Over the 3 month period, 43 males and 32 females participated in the survey. Their average age was 75 ± 9 years.

Reason for diuretic

Most patients were being prescribed diuretics for major indications such as congestive heart failure, pulmonary oedema and renal disease. In several cases the diuretics were also prescribed for hypertension and ankle oedema not caused by heart failure or renal insufficiency.

Frusemide

Sixty six out of the 75 patients were taking frusemide. Fifty of these patients were taking the drug as a single early morning oral dose and in 96% of these cases the dose was 80 mg or less. 13 patients were receiving divided doses of oral frusemide, an early morning

dose and an equivalent or lower dose given at either 2, 4, or 6 pm. The remaining 3 patients were receiving intravenous injections of frusemide.

Bumetanide

Only 9 patients were taking bumetanide. Five were taking a single oral dose and 4 were taking bumetanide twice daily. Three patients who were being prescribed bumetanide at home were prescribed an equivalent dose of frusemide while in hospital.

Dose changes in diuretic since admission

Nine patients who had been admitted with pulmonary oedema and congestive heart failure received intravenous frusemide before being prescribed oral doses. In one of these patients the intravenous dose was administered as an infusion over 1 h. In order to obtain adequate diuresis, several patients also had their oral dose of diuretic increased while in hospital.

Other drugs

Most patients were being prescribed several other drugs and in the majority of cases at least one of the drugs was being taken at the same time as frusemide.

Time of diuretic administration in relation to breakfast

In the medical wards of the Edinburgh Royal Infirmary the timing of breakfast often coincided with the early morning drug round. The times of administration of frusemide and bumetanide in relation to hospital breakfast are shown in Fig 5.1. On average each

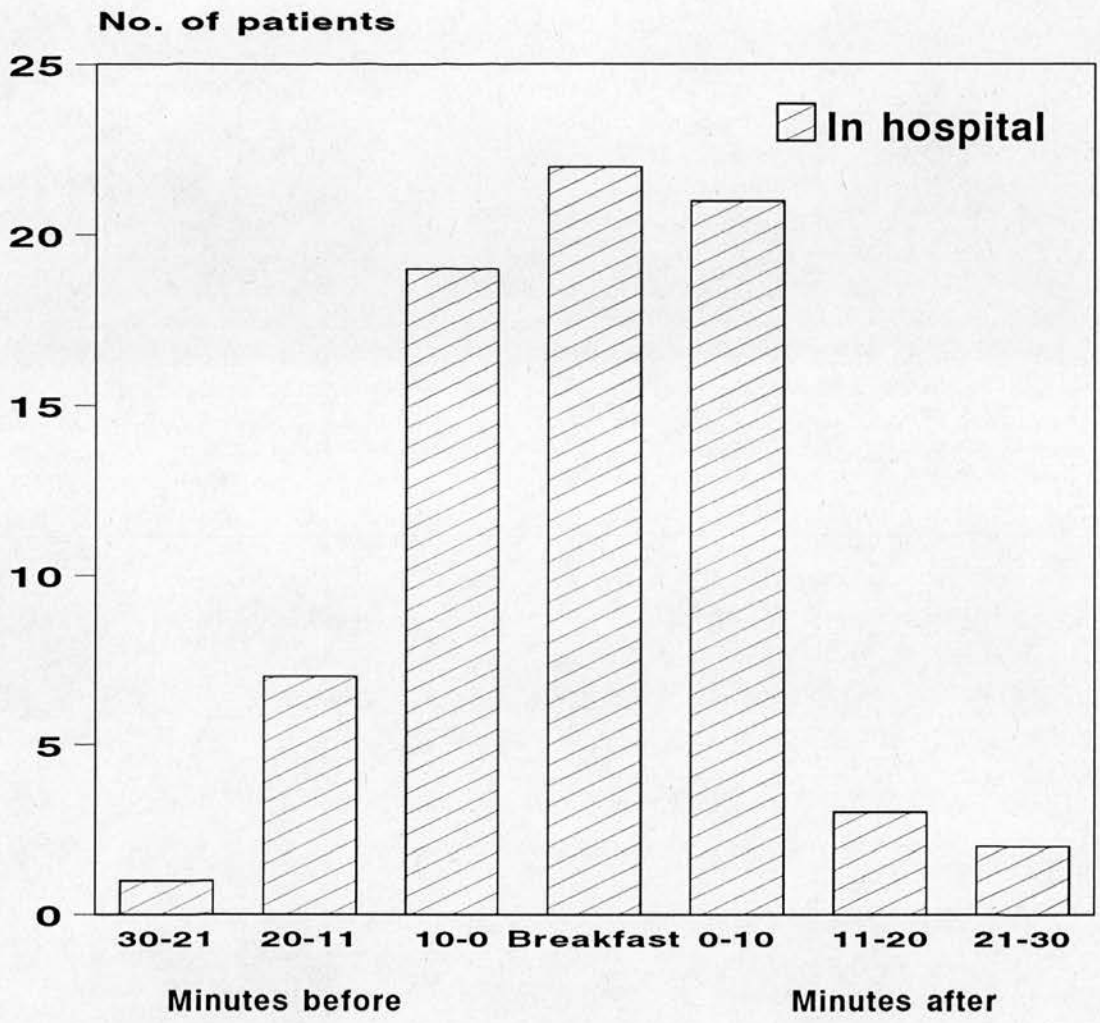


Figure 5.1 Time of diuretic administration in relation to breakfast in 72 hospital patients admitted to wards 23 and 24.

patient took 10 - 15 minutes to eat their breakfast which usually consisted of porridge or cereal and toast or a roll with butter and jam. All of the 72 patients taking an oral dose of diuretic had taken it within ± 30 minutes of breakfast and 83 % had taken the drug either during breakfast or within ± 10 minutes of the start or finish of breakfast.

The patients were also asked when they normally took their diuretic at home in relation to breakfast (See Fig 5.2). A similar pattern emerged with 74 % taking their diuretic either with breakfast or within ± 10 minutes of breakfast. However a larger proportion of patients did seem to take frusemide before breakfast when at home.

Overall, the majority of patients surveyed were taking a single oral dose of diuretic (usually frusemide) with or very close to hospital breakfast. Since the absorption of frusemide (and to a lesser extent bumetanide) has been shown to be grossly impaired when administered with food in healthy volunteers, the survey therefore shows the extent to which these drugs are taken in circumstances where reduced absorption may seriously interfere with efficacy.

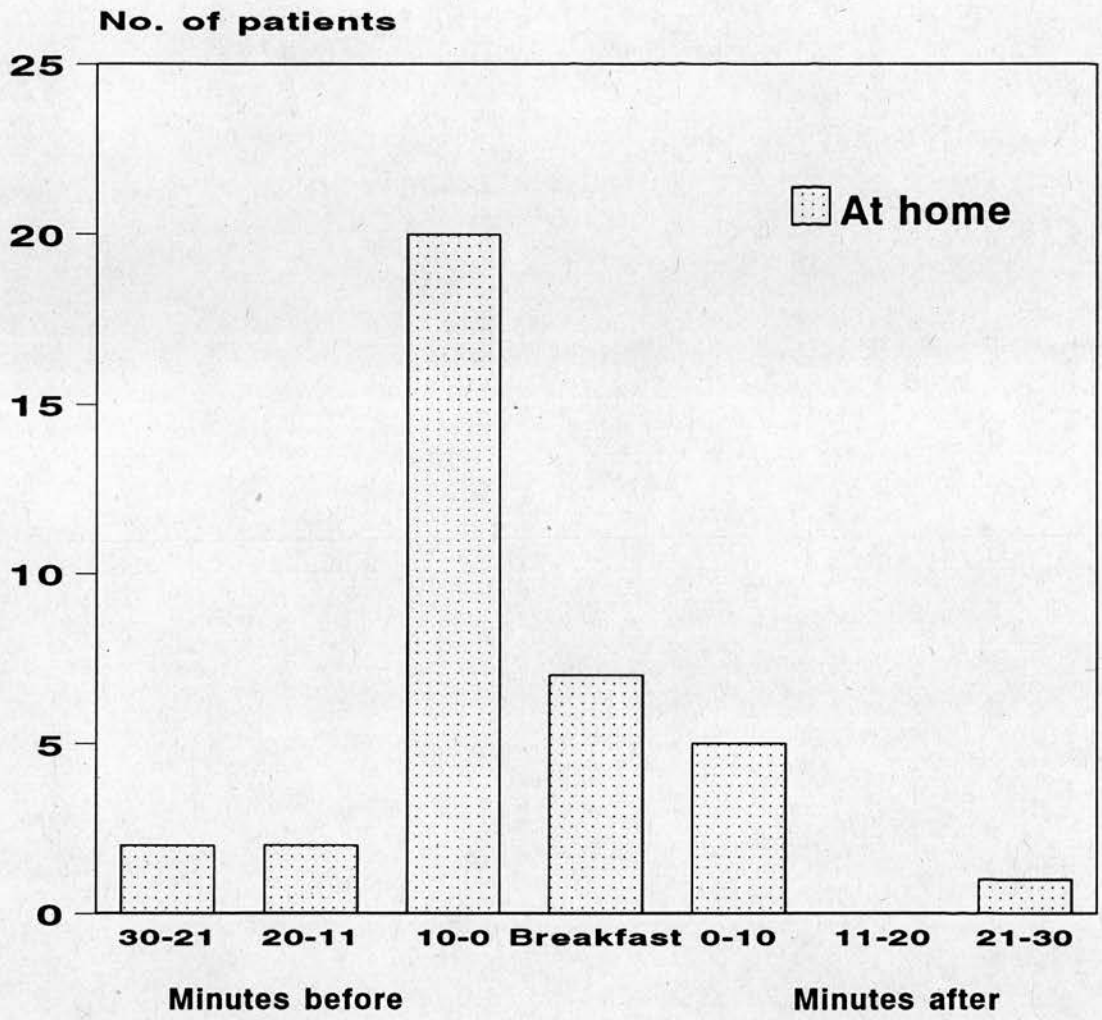


Figure 5.2 Time of diuretic administration at home in relation to breakfast.

CHAPTER 6

INFLUENCE OF HOSPITAL BREAKFAST ON THE ABSORPTION AND EFFICACY OF FRUSEMIDE

Section 6.1. Introduction

Most pharmacokinetic studies involve healthy, young subjects given single doses of drugs under ideal/controlled fasting conditions. In practice however, most drugs are administered under nonfasting conditions to unhealthy elderly patients treated on a long term basis.

Frusemide is widely used and it has previously been shown that its oral absorption is reduced by approximately 30 % when given to healthy volunteers with food. Also, the survey showed that it is given to hospital patients in a random manner often with or in close proximity to breakfast. It is therefore taken in a situation which could potentially alter its absorption and hence the diuretic response.

The aim of this study was to establish whether the absorption and effect of frusemide was improved in hospital patients when the time of drug administration in relation to meals was controlled. This information could lead to the more effective clinical use of frusemide.

Section 6.2. Methods

Subjects

Ten medical inpatients (6 male and 4 female) aged 55 to 77 years (average 70 ± 7 yrs) from wards 23 and 24 of the Edinburgh Royal Infirmary were studied after giving informed verbal consent. The details of the patients are given in Table 6.1. They were given an information sheet which included a full description of the objective and plan of

Table 6.1. Patient details

Patient	Age & Sex	Daily Dose of Frusemide (mg)	Medical Diagnosis	Other Medication
1	67 M	80	Exacerbation of chronic obstructive airways disease	Diazepam 5mg at night Dihydrocodeine 60mg bd Glipizide 10mg every morning Innovace 20mg at night Isosorbide mononitrate 60mg Aminophylline 225mg bd Senna 2 tablets at night Tildiazem Retard 90mg bd
2	60 M	40	Atrial fibrillation	Aspirin 75mg every morning Digoxin 125mg every morning Diltiazem SR 90mg bd Enalapril 10mg at night Isosorbide mononitrate 40mg bd
3	71 M	80	Myocardial infarction	Aspirin 75mg every morning Enalapril 20mg at night Isosorbide mononitrate 20mg bd Senna 2 tablets at night
4	55 M	80	Alcoholic cardiomyopathy	Allopurinol 300mg every morning Co-proxamol 2 tabs 6-8 hourly Enalapril 20mg at night
5	77 F	80	Left vetricular failure	Digoxin 0.125mg every morning Enalapril 10mg every morning Erythromycin 250mg qid Isosorbide mononitrate 20mg bd Senna 2 tablets at night
6	70 F	40	Left ventricular failure	Aspirin 150mg every morning Diltiazem SR 90mg bd Enalapril 20mg at night Isosorbide mononitrate 10mg bd Ranitidine 300mg bd

Table 6.1. Continued.

Patient	Age & Sex	Daily Dose of Frusemide (mg)	Medical Diagnosis	Other Medication
7	74 F	120	Left ventricular failure	Aspirin 75mg every morning Enalapril 20mg every morning Paracetamol 1g 6 hourly Senna 2 tablets at night
8	74 M	80	Left ventricular failure	Aspirin 75mg every morning Enalapril 20mg at night Isosorbide mononitrate 10mg bd Paracetamol 1g 4-6 hourly
9	73 F	80	Chest tightness	Aspirin 300mg every morning Enalapril 20mg at night Isosorbide mononitrate 60mg bd
10	76 M	80	Left ventricular failure	Dipyridamole 25mg tid Enalapril 10mg at night Salbutamol 2mg bd

Note: bd = twice daily; tid = thrice daily; qid = four times daily

the study and the study was approved by the Lothian Health Board Medicine and Clinical Oncology Research Ethics Sub-Committee.

All patients were receiving routinely a single oral dose of at least 40 mg frusemide, in the morning, while in hospital. They were receiving no other diuretic agents (e.g amiloride). Patients were maintained on their normal doses of other drugs at the usual times throughout the study. Any patients with impaired renal function (judged from creatinine clearance) other than that due to cardiac failure and age were excluded from the study. Patients with any acute illness (e.g. pneumonia) were also excluded.

Procedure

A randomised design was used. Each patient was studied on 2 consecutive days. On one occasion the patient received his/her oral dose of frusemide as normal during the early morning drug round (uncontrolled administration) and the time in relation to breakfast was recorded. On the other study day the time of the frusemide dose was controlled in relation to the time of breakfast. Frusemide was given 2 h after food intake (controlled administration). The hospital breakfast consisted of porridge or cereal and a roll or toast with butter and jam/marmalade. On both study days the patients ate lunch and dinner as usual.

On each occasion venous blood samples (10 ml) were taken via an indwelling cannula immediately before and 15, 30, 45, 60, 90, 120, 150, 180, 240, 360 and 480 min after frusemide administration. All samples were collected in lithium heparin tubes and centrifuged at 3000 rpm for 15 min. Plasma was stored at -20^{0}C.

Patients emptied their bladders just before taking their dose of frusemide. Urine was then collected from 0-2, 2-4, 4-6 and 6-8 h after dosing. Urine volumes were recorded and aliquots stored at -20^0 until analysis.

Analysis of samples

Frusemide concentrations in plasma and urine were measured by high performance liquid chromatography as described in Chapter 2. No interfering peaks from other drugs were found in any of the patients plasma or urine samples. Urinary sodium and potassium were analysed by ion specific electrodes.

Pharmacokinetic analysis

Due to the irregular absorption patterns of frusemide in most patients, plasma concentration time curves were not fitted to the pharmacokinetic model by computer. Instead, the $AUC_{0\text{-}8h}$ were calculated using the trapezoidal rule (Equation 2.3).

Statistics

Statistical comparisons were made using the non-parametric Wilcoxon signed-ranks test and values of <0.05 were accepted as significant.

Section 6.3. Results

The ten patients took their dose of frusemide within $\pm$ 25 minutes of breakfast (See Table 6.2). The individual plasma concentration time curves for controlled and uncontrolled frusemide administration are shown in Fig 6.1. Out of the 10 patients, 7 were taking

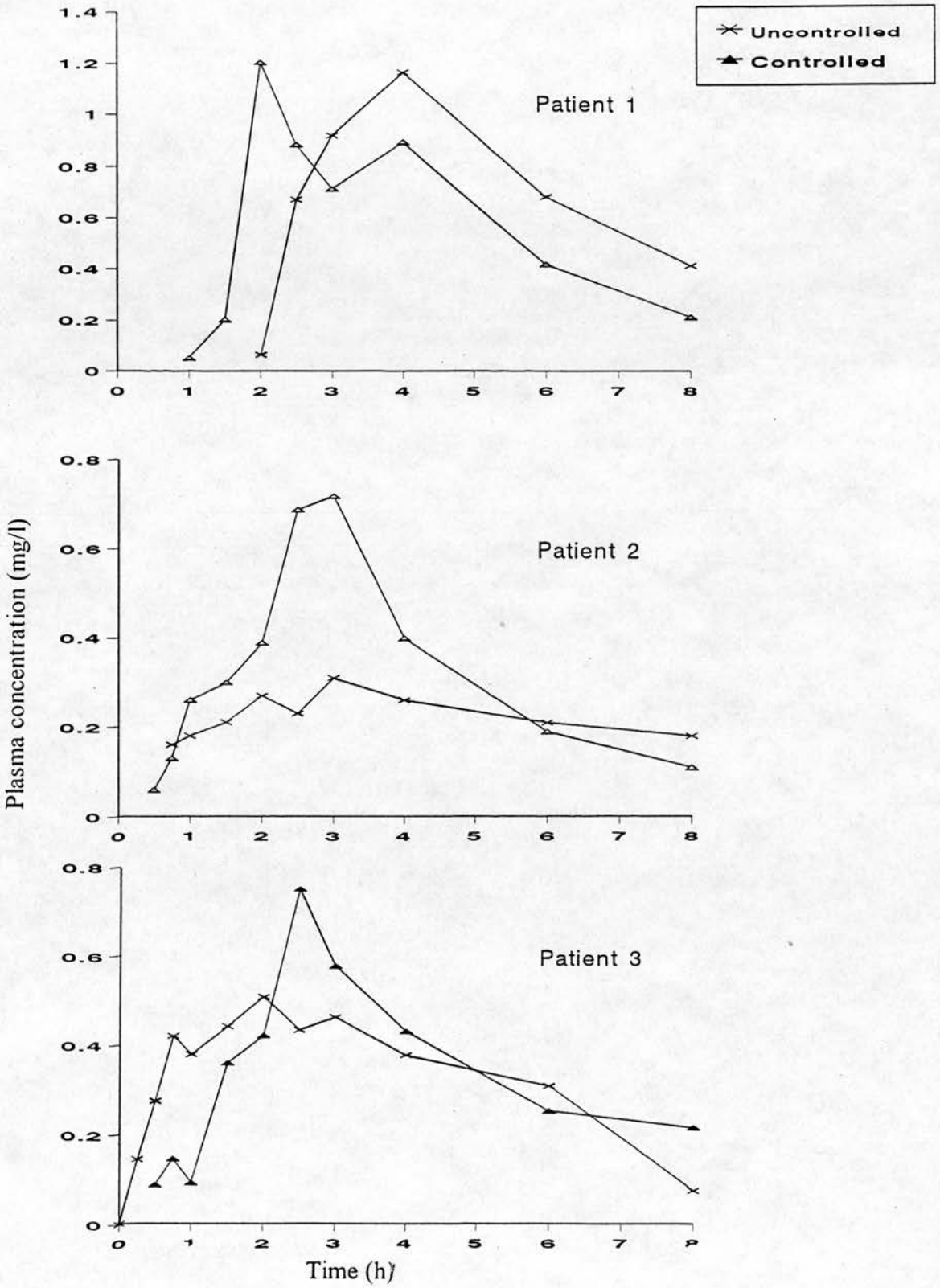
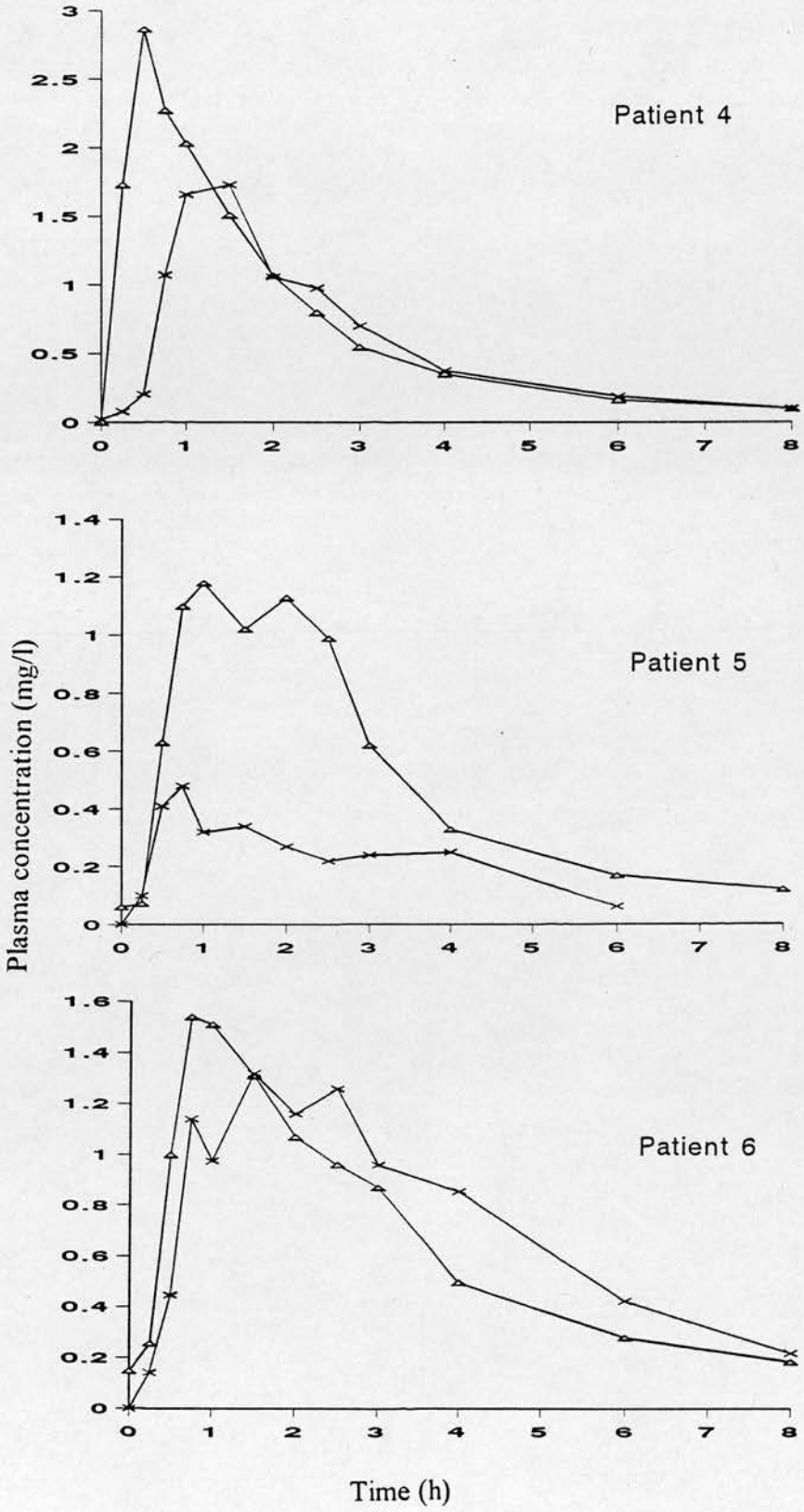


Figure 6.1. Individual plasma concentration versus time curves following uncontrolled and controlled administration of frusemide to 10 hospital patients.



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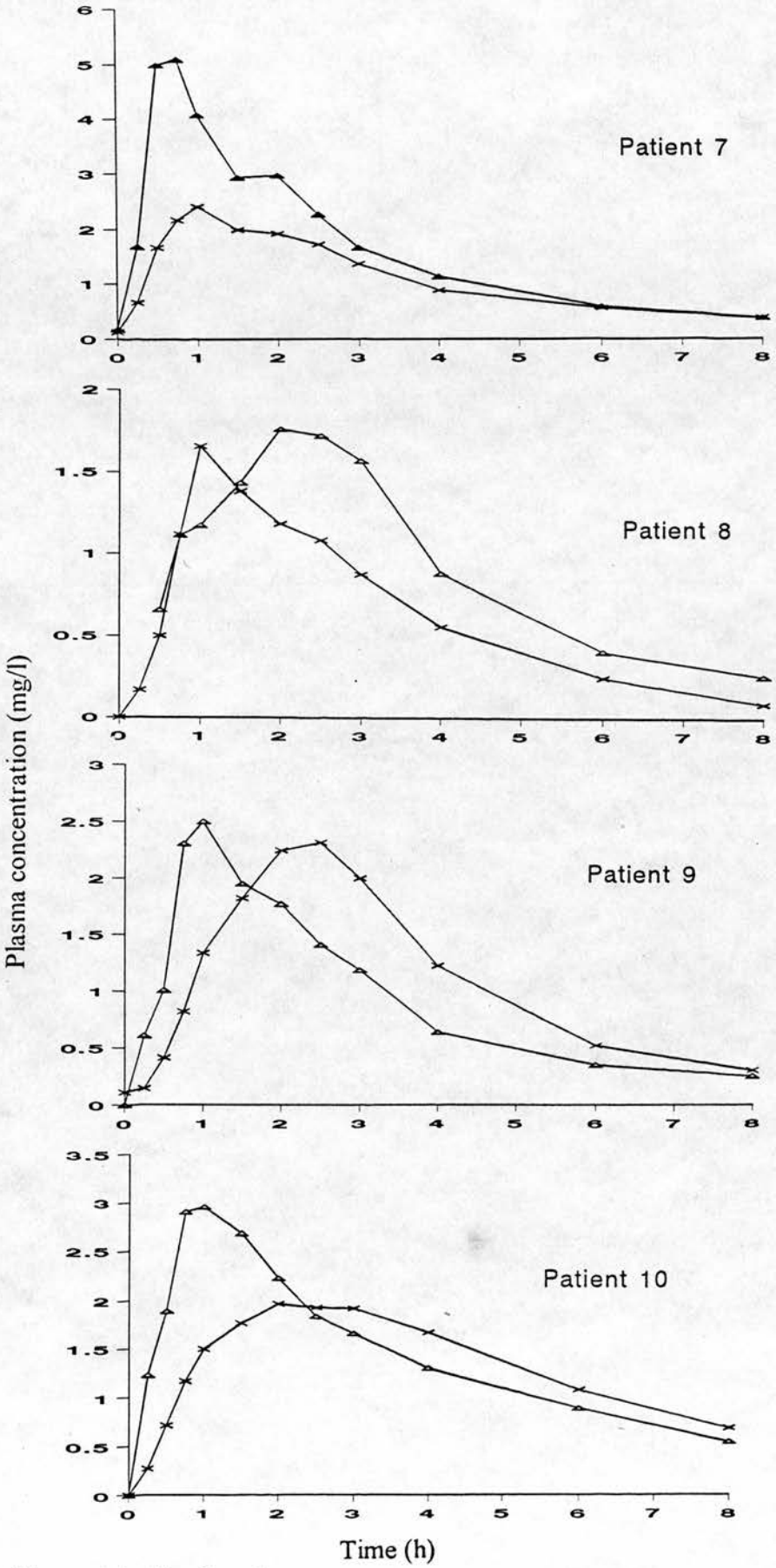

Figure 6.1. Continued.

80 mg of frusemide. The remaining 3 patients (numbers 2, 6 and 7) were taking 40, 40 and 120mg, respectively. The plasma concentrations obtained from these 3 patients were normalised to 80mg so that all the results were comparable. The resulting mean plasma concentration time curves are shown in Fig 6.2.

Cmax, Tmax and AUC_{0-8h} values are given in Table 6.2. In all patients peak plasma concentrations were higher following controlled administration ($P < 0.05$, compared to uncontrolled administration). The time to peak concentration was reduced from 1.92 ± 1.01 h following uncontrolled administration to 1.45 ± 0.86 h following controlled administration. The Tmax values were however not significantly different. Mean AUC_{0-2h} and AUC_{0-4h} values following controlled administration (2.78 ± 1.52 and 4.77 ± 2.16 mg.h/l, respectively) were significantly greater ($P<0.05$) compared to uncontrolled administration (1.85 ± 0.96 and 3.75 ± 2.19 mg.h/l). The AUC_{0-8h} were greater following controlled administration of frusemide in subjects 2, 4, 5, 7, 8 and 10. However, the overall mean AUC_{0-8h} values for controlled and uncontrolled administration were not significantly different.

Renal elimination

The recoveries of frusemide after controlled and uncontrolled administration are shown in Fig 6.3 and Table 6.3. Over the first 2 h the percentage of the frusemide dose excreted unchanged was significantly higher following the administration of frusemide 2 h after breakfast ($P < 0.05$). From 2-4 and 4-8 h the differences were not significant compared to uncontrolled administration. Total urinary recovery of unchanged frusemide was higher following controlled administration in subjects 2, 4, 5, 7, 8 and 10 which

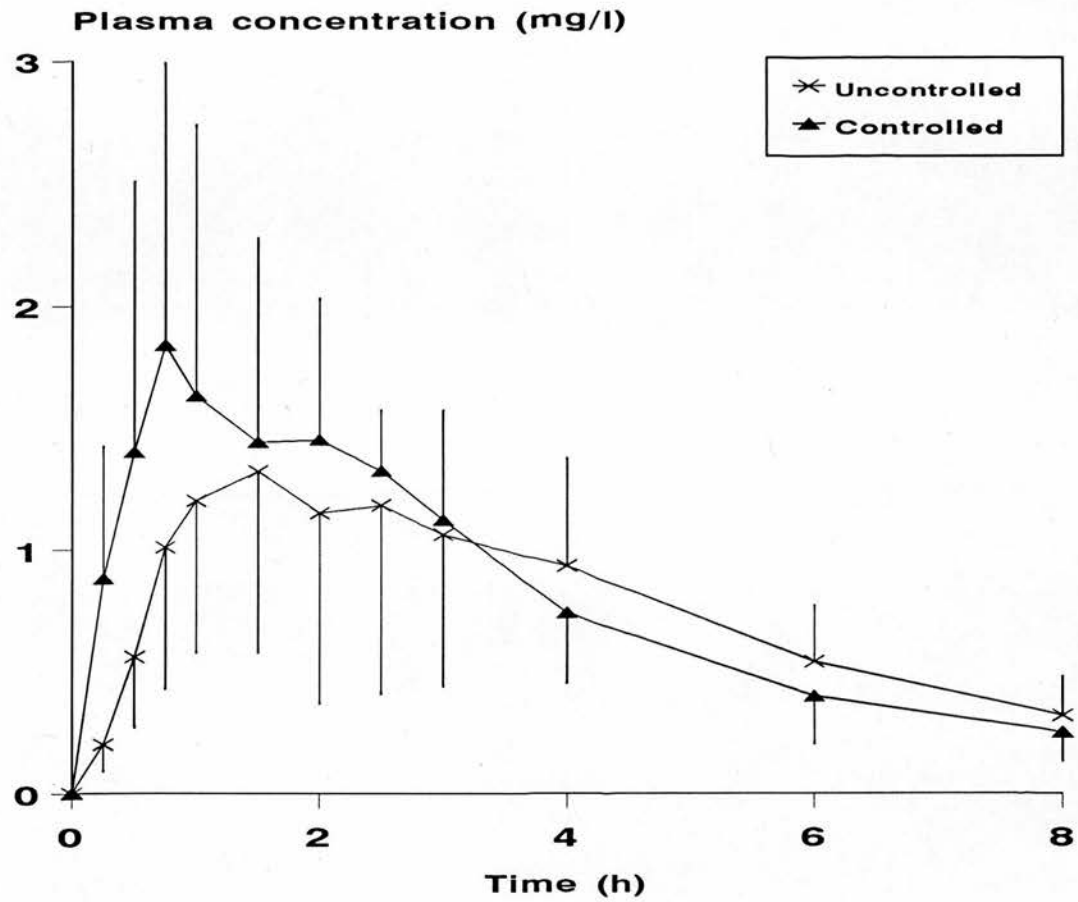

Figure 6.2 Mean plasma concentration versus time curves following uncontrolled and controlled administration of frusemide to 10 hospital patients. Bars = ± s.d.

Table 6.2. Peak concentrations (Cmax), time to peak concentrations (Tmax) and area under the plasma concentration time curves following uncontrolled and controlled administration of frusemide to 10 hospital patients.

Patient	Time of dose in relation to breakfast (uncontrolled day)	Content of breakfast	Cmax (mg/l)		Tmax (h)		AUC_{0-8h} (mg.h/l)	
			Uncontrolled	Controlled	Uncontrolled	Controlled	Uncontrolled	Controlled
1	With breakfast	Shredded wheat,roll + butter & jam	1.17	1.21	4.00	2.00	4.59	4.10
2	8 min. before	Cornflakes,roll + butter & jam	0.62	1.44	3.00	3.00	3.34	4.92
3	2 min. before	Cornflakes,toast + butter & jam	0.51	0.75	2.00	2.50	2.65	2.64
4	Straight after	Cornflakes,toast + butter & jam	1.73	2.86	1.50	0.50	4.41	5.61
5	4 min. before	Cornflakes,banana, toast + butter	0.48	1.18	0.75	1.00	1.49	4.02
6	20 min. after	Porridge with milk,toast + butter	2.64	3.08	1.50	0.75	11.48	10.20
7	3 min. before	Porridge,toast + butter & marmalade	1.61	3.41	1.00	0.75	5.82	8.75
8	14 min. before	Cornflakes, toast + butter & jam	1.66	1.77	1.00	2.00	4.99	6.96
9	23 min. after	Cornflakes, banana, toast + butter	2.32	2.50	2.50	1.00	8.72	7.29
10	With breakfast	Cornflakes, toast + butter & jam	1.97	2.96	2.00	1.00	10.72	11.51
		Mean	1.47	2.12	1.92	1.45	5.82	6.60
		± s.d.	0.76	0.95	1.01	0.86	3.39	2.88
		P	<0.05		NS		NS	

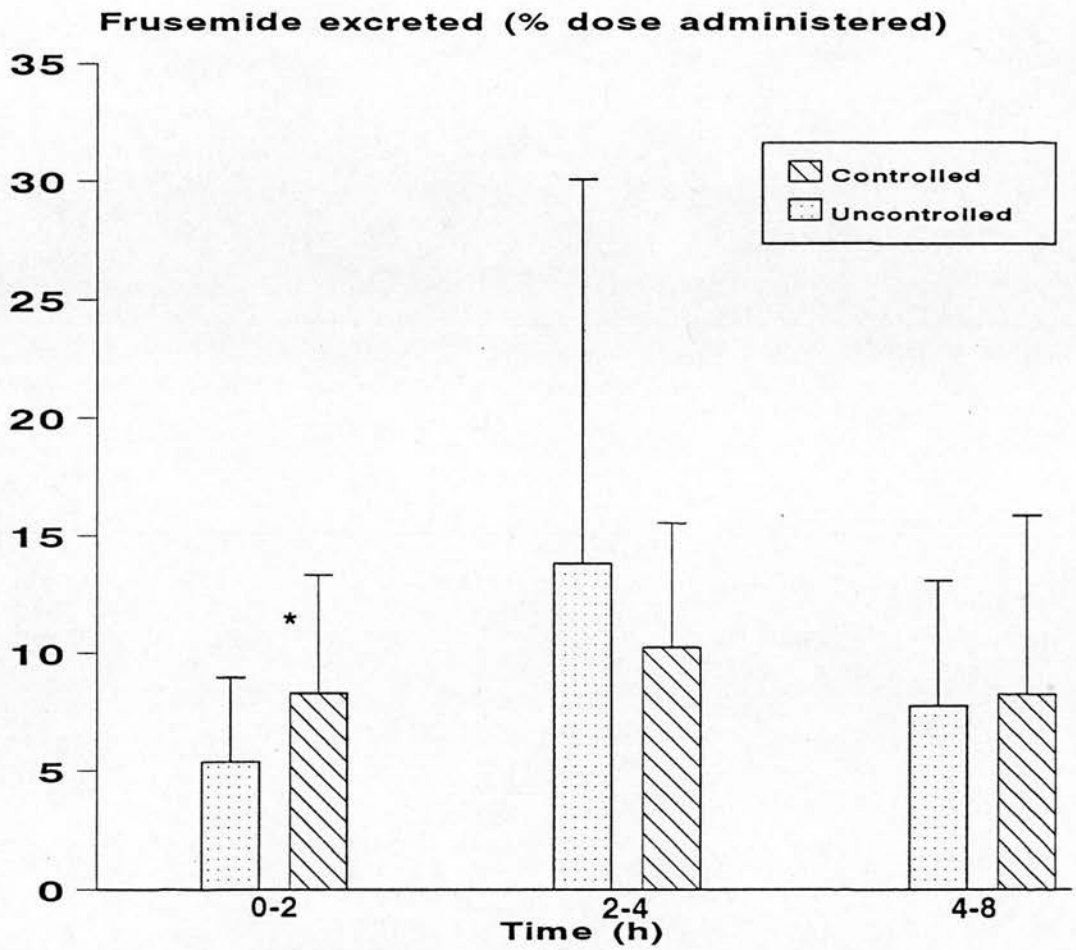

Figure 6.3. Urinary recoveries of frusemide (% of administered dose) following controlled and uncontrolled administration in 10 hospital patients. *P<0.05, compard to uncontrolled administration.

Table 6.3. Urinary recovery of frusemide over 8 h (% of administered dose) following uncontrolled and controlled administration to 10 hospital patients.

Patient	Urinary recovery (% of administered dose) of unchanged frusemide in 8h	
	Uncontrolled	Controlled
1	27.1	13.4
2	17.5	24.2
3	12.8	8.0
4	6.9	13.5
5	13.0	21.0
6	46.2	35.8
7	19.8	31.2
8	20.1	34.0
9	36.2	26.6
10	22.0	27.0
Mean	22.2	23.5
±s.d.	11.7	9.4

corresponds with the greater $AUC_{0\text{-}8h}$ values obtained for these subjects. However the mean total urinary recoveries for controlled and uncontrolled frusemide administration were not significantly different.

Response to frusemide

Urine volumes and sodium excretion following controlled and uncontrolled administration were compared by ratio (i.e. uncontrolled / controlled (U/C).

Urine volume

The average 8 h urinary volume ratio (U/C) was 0.99 ± 0.57. However over the first 2 h following frusemide administration the U/C ratio for urine volume was 0.72 ± 0.40, indicating a greater response after controlled administration. However the 95% confidence interval (95% CI) for the mean value did contain 1.0 (0.39 to 1.01). The ratios from 2-4 and 4-8 h were 1.32 ± 0.91 (95% CI = 0.62 to 2.02) and 1.36 ± 1.31 (95% CI = 0.36 to 2.37), respectively.

Sodium excretion

A similar pattern was found for sodium excretion. From 0-2 h the sodium excretion ratio was 0.73 ± 0.41 (95% CI= 0.41 to 1.03). From 2-4 and 4-8 h the ratios were 1.72 ± 1.58 (95% CI = 0.40 to 3.05) and 1.49 ± 1.03 (95% CI = 0.63 to 2.35). The mean 8 h sodium excretion ratio was 1.14 ± 1.13, which was not significantly different from 1.0.

Section 6.4. Discussion

The pharmacokinetics and pharmacodynamics of frusemide have previously been investigated in healthy elderly subjects and patients with different pathology. Chaudry *et al* (1984) studied the effects of intravenous frusemide on urine volume and sodium excretion in healthy young and healthy elderly subjects under fasting conditions. Total 24 h response to frusemide showed no difference in the two groups, however the pattern of response was different. Peak effect on sodium and water excretion was delayed and reduced in the elderly subjects but the response was prolonged. Kerremans *et al* (1983) carried out a similar study using intravenous and oral frusemide in aged patients with varying diagnoses. All the patients had used frusemide for at least three months and most used other drugs which were continued during the study. After frusemide administration the patients were allowed to eat and drink as usual. Under these conditions, the absorption of frusemide and its volume of distribution were virtually unchanged compared to results obtained from healthy volunteers. Total plasma clearance and renal clearance were however reduced leading to increased plasma levels and AUC. In spite of this a reduction in the renal elimination of frusemide was demonstrated together with a decreased response. Studies by Brater *et al* (1982a) and Greither *et al* (1984) showed little if any quantitative change in frusemide absorption in hospital patients with heart failure compared to normal subjects. However Brater *et al* (1984) subsequently showed delayed absorption of frusemide in heart failure patients causing attainment of peak urinary excretion rates of diuretic two- to threefold lower than in normal subjects. The pharmacodynamics of frusemide were also altered as demonstrated by a blunting of the dose response relationship.

In elderly patients, the response to frusemide may already be altered or diminished partly by age and partly by disease. The presence of food is an added variable which could further reduce frusemide absorption and response. In healthy volunteers, Hammarlund *et al* (1984) found that food significantly delayed the absorption of frusemide by approximately 60 minutes. Beermann and Midskov (1986) found a 30 % decrease in frusemide bioavailability when administered with breakfast. We also found that the bioavailability of frusemide was reduced significantly when given to healthy volunteers with breakfast and our survey showed that the majority of patients took it with or close to the time of breakfast.

In the present study administration of frusemide 2 h after breakfast produced significantly higher peak plasma concentrations and tended to reduce the time to the peak. $AUC_{0\text{-}2h}$ and $AUC_{0\text{-}4h}$ were significantly greater following controlled administration. The percentage of the dose excreted unchanged in the urine was also significantly higher over the first 2 h following controlled administration. Over the same period the natriuretic and diuretic response to frusemide was also generally higher compared to uncontrolled administration although the result was not statistically significant.

These results must be interpreted with caution since several other factors, in addition to food, may be responsible for the changes described above. The time of frusemide administration itself may have altered the time course of elimination and response. During uncontrolled administration, frusemide was usually taken around 08.00 h, hence the dose given 2 h after breakfast was given at approximately 10.00 h. Investigations in

both animals and healthy adults have implicated possible diurnal variation in diuretic, natriuretic and kaliuretic effects of frusemide (Hemal *et al.* 1988; Tateishi *et al.* 1988). Sodium and water excretion was increased 2 h postadministration when frusemide was administered at 0700 h as opposed to 1900 h. The authors hypothesised a difference in frusemide excretion secondary to diurnal variation in frusemide metabolism, tubular secretion and/or protein binding as a potential cause of the observed variation. It is also possible that the differences merely reflected day to day variation in frusemide absorption and response. The kinetics and the dynamics of frusemide should have been studied on two separate occasions following controlled administration in order to determine any influence of daily variation. Finally, when frusemide was administered 2 h after breakfast the drug was also administered 2 h after the patients had taken their other medication. This reduced the chance of not only food-drug interactions but also drug-drug interactions. For example, coadministration of nonsteroidal anti-inflammatory drugs with frusemide has been shown to reduce the natriuretic response (Daskalopoulos *et al.* 1985; Patak *et al.* 1975; Planas *et al.* 1983).

Overall however, we found no significant improvement in either $AUC_{0\text{-}8h}$, urinary recovery of frusemide or total natriuretic or diuretic response over 8 h when frusemide was administered 2 h after breakfast. Therefore, despite our findings in healthy volunteers where food produced, on average, a 30 % reduction in the bioavailability of frusemide, it seems that in everyday clinical practice frusemide absorption may not be altered by breakfast to an extent where drug efficacy might be compromised.

CHAPTER 7

EFFECTS OF DOSE ON THE ABSORPTION AND CLEARANCE OF FRUSEMIDE

Section 7.1. Introduction

Despite the widespread use of frusemide, little is known about the mechanism of its absorption from the gastrointestinal tract in man. However evidence from animal studies suggests that it may exhibit site specific absorption. Chungi *et al* (1979) on administering buffered solutions of frusemide to different gastrointestinal sites of the rat gut found rapid absorption from the stomach and slower absorption from the small intestine. In a similar study carried out by Ritschel *et al* (1991) frusemide was also shown to have an absorption window comprised of the upper gastrointestinal tract. In addition they also found that an active transport mechanism may be partly involved in its absorption, which is a saturable process (Rowland and Tozer, 1980). If this is also the case in humans, it is possible that the absorption of frusemide may be dose-dependent.

Evidence of dose-dependency is usually assessed by increasing the dose of a drug and after normalising the resultant plasma concentrations or amounts excreted to the dose administered, observing whether such values correspond. When the values do not correspond, dose-dependency is inferred.

In the following study we have therefore compared the absorption and elimination of frusemide administered as an oral solution over the dosage range 10 - 80 mg in healthy volunteers.

Section 7.2. Methods

Eight healthy males, aged 21-38 years (average 28 ± 5 yrs.) and weighing 51-83 kg (average 70 ± 10kg) took part in the study. The volunteers were healthy according to

history, clinical examination and haematological and biochemical tests. They were negative for Hepatitis B and HIV infection. The protocol was approved by the Lothian Health Board Healthy Volunteers Studies Ethics of Medical Research Sub-Committee and all volunteers gave informed written consent after the aim and procedure had been explained.

No other medication was allowed for 1 week before and during the study. Alcohol was prohibited from 24 h prior to and during the study. All the volunteers fasted from 10.00 pm. the night before each study day.

Procedure

"Lasix" injection solution (frusemide 10 mg/ml) was administered orally as 1ml (10 mg), 4 ml (40 mg) or 8 ml (80 mg) diluted to a final volume of 100 ml with orange squash (Kia-Ora). The container was rinsed with a further 100 ml of water and this was also administered to the volunteer.

To compensate for fluid loss during the study, 500 ml of 0.9 % sodium chloride was administered intravenously over the first hour. For the remainder of the study urine volumes were replaced by a similar volume of 5 % dextrose again administered intravenously.

The volunteers remained supine during the study and had nothing to eat or drink up to 5 h after dosing. A light lunch was provided between 5 and 6 h.

Blood Samples

Blood samples were collected through an indwelling cannula placed in a forearm vein. Samples (10 ml) were taken just before dosing and at 10, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180 minutes and hourly for the next 5 h after drug administration. All the samples were collected in lithium heparin tubes and centrifuged at 3000 rpm for 15 min. Plasma was stored at approximately -20^0.

Urine collection

The volunteers emptied their bladders prior to drug administration. Urine was then collected at 1, 2, 3, 4, 5, 6 and 8 h after drug administration. The volunteers also collected urine from 8 - 24 h at home. Urine volumes were recorded and an aliquot frozen at -20^0 until assayed.

Analysis of samples

Frusemide concentrations were determined by high performance liquid chromatography using fluorescence detection as described in Chapter 2.

Data Analysis

Plasma concentration time curves were fitted to a one compartment model with 2 phases (Equation 2.12), using the "Siphar" curve fitting and modelling program. Renal clearance (CL_R) values were calculated using Equation 2.9. Peak plasma concentrations (Cmax) and time to peak plasma concentrations (Tmax) were observed from the measured plasma concentrations following drug administration.

The $AUC_{0-\infty}$, Cmax and Ae (total amount excreted unchanged in the urine from 0 - 24 h) were normalised for dose by multiplying the values by 4 for the 10 mg dose and dividing by 2 for the 80 mg dose.

Analysis of variance was used to make statistical evaluation of the data. A P value of less than 0.05 was accepted as evidence of a statistically significant difference.

Section 7.3. Results

Mean concentrations of frusemide in plasma resulting from the administration of 10, 40 and 80 mg are shown graphically in Fig 7.1. Normalised plasma concentrations are shown in Fig 7.2. The resulting pharmacokinetic parameters obtained from the three administered doses are given in Table 7.1. Dose normalised maximum plasma concentrations were not significantly different for all three doses. The mean Tmax for the 40 mg dose was 40 minutes. No significant difference was observed among the doses although there was a tendency towards increasing Tmax values with increasing dose. Dose normalised $AUC_{0-\infty}$ values were also not significantly different (Table 7.1).

The total mean amounts of frusemide excreted unchanged in the urine in 24 h were 4.1, 18.0 and 38.0 mg for the 10, 40 and 80 mg doses, respectively. Cumulative amounts of frusemide excreted are shown in Fig 7.3. When the values were normalised for dose no significant differences were found (Fig 7.4 and Table 7.1).

The renal clearances for the 10, 40 and 80 mg doses were 106.7 ± 39.8, 90.5 ± 32.0 and 99.0 ± 33.2 ml/min. These values were not significantly different. Hourly renal clearances of frusemide for all the doses were constant over 8 h (See Fig 7.5).

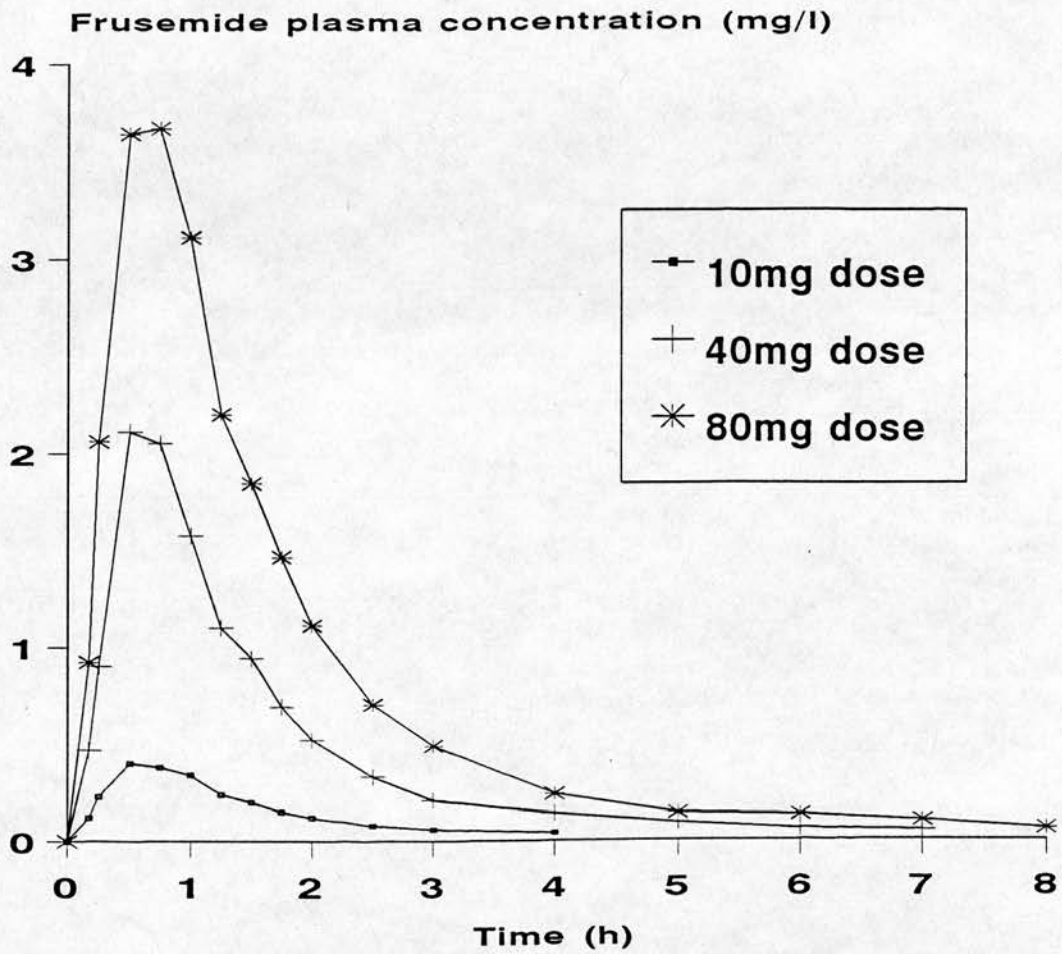

Figure 7.1. Mean plasma concentrations of frusemide in 8 healthy volunteers following the oral administration of 10, 40 and 80 mg.

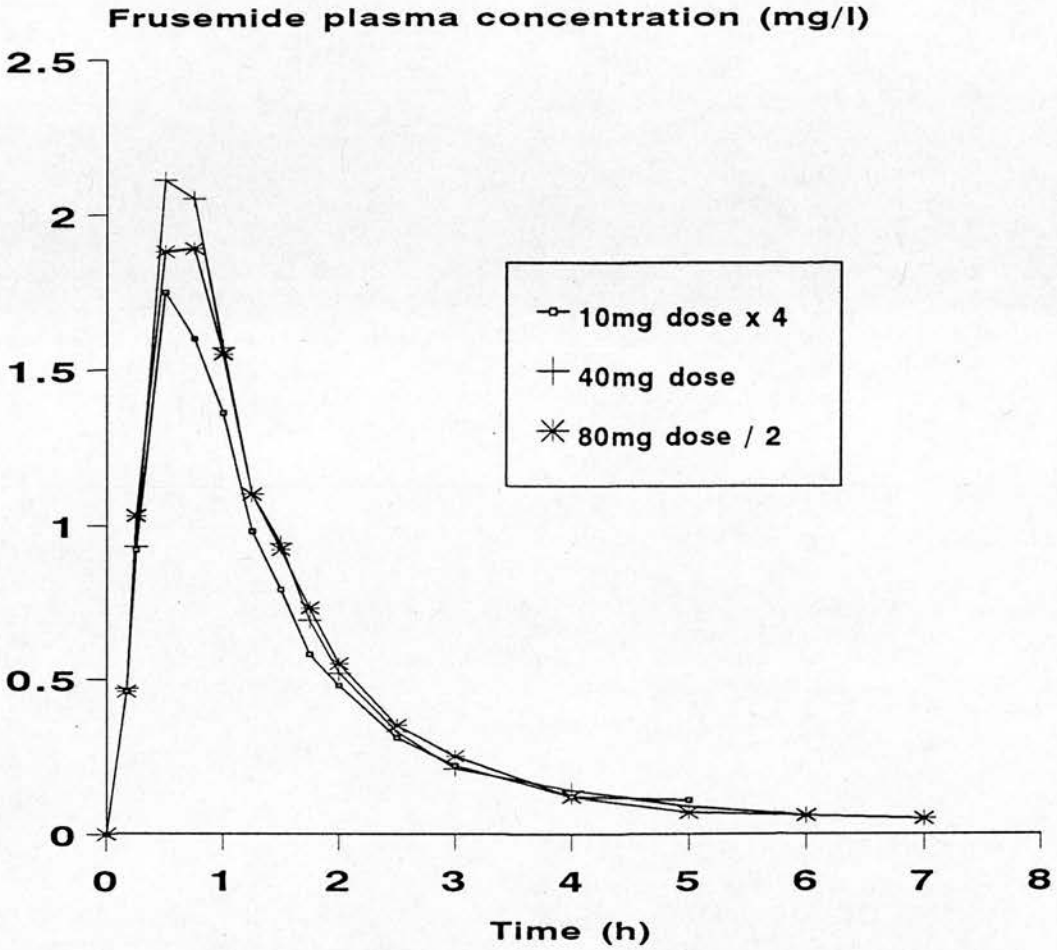

Figure 7.2. Mean plasma concentrations of frusemide normalised to the 40mg dose after administration of 10, 40 and 80 mg to 8 healthy volunteers.

Table 7.1. Mean (± s.d.) pharmacokinetic parameters following administration of frusemide 10mg, 40mg and 80mg to 8 healthy volunteers.

Dose of Frusemide	Cmax (mg/l)	Tmax (h)	$AUC_{0-\infty}$ (mg.h/l)	Ae (mg)	CL_R (ml/min)
10mg	0.47 ± 0.16	0.56 ± 0.22	0.71 ± 0.28	4.06 ± 1.29	106.7 ± 39.8
40mg	2.33 ± 0.54	0.65 ± 0.23	3.54 ± 0.88	18.02 ± 3.37	90.5 ± 32.0
80mg	4.27 ± 1.19	0.78 ± 0.25	6.81 ± 1.68	38.01 ± 4.22	99.0 ± 33.2
Dose normalised 10mg	1.88 ± 0.63		2.84 ± 1.13	16.24 ± 5.19	
Dose normalised 80mg	2.13 ± 0.60		3.40 ± 0.84	19.01 ± 2.11	

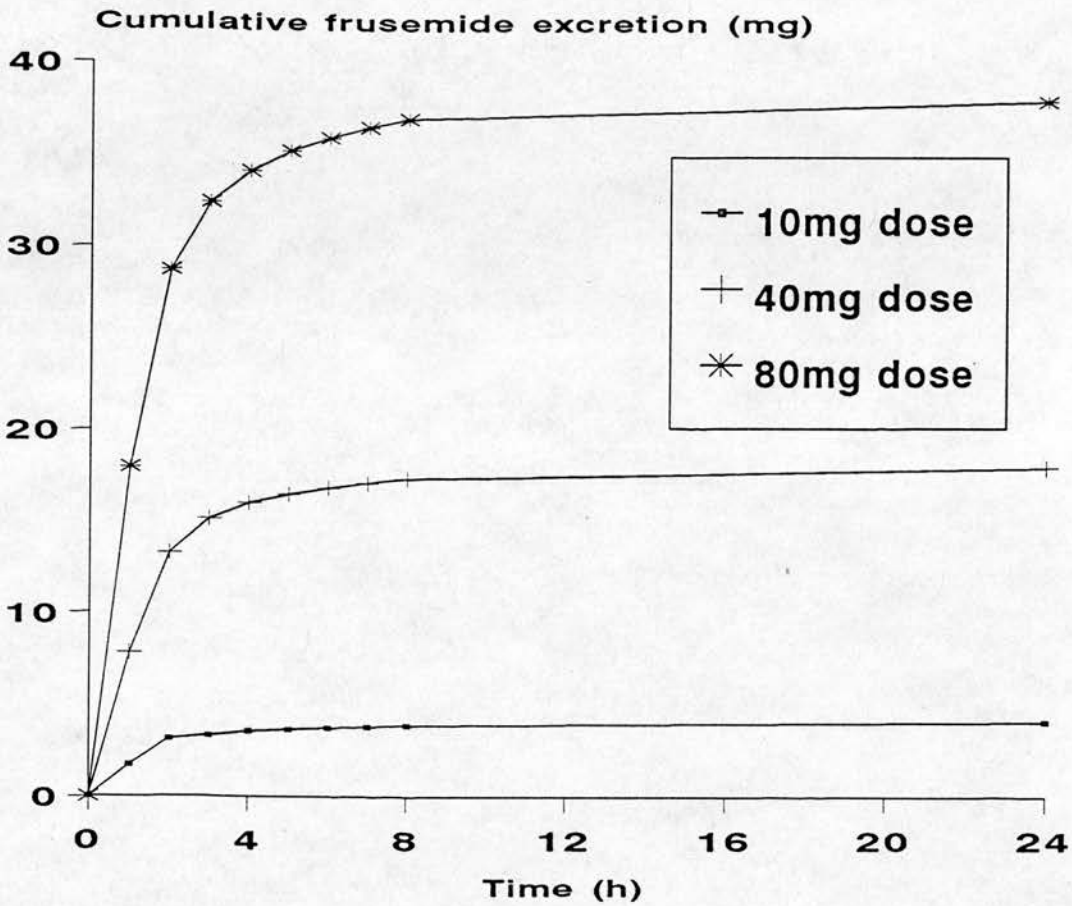

Figure 7.3. Mean cumulative amounts of frusemide excreted in the urine following administration of 10, 40 and 80 mg to 8 healthy volunteers.

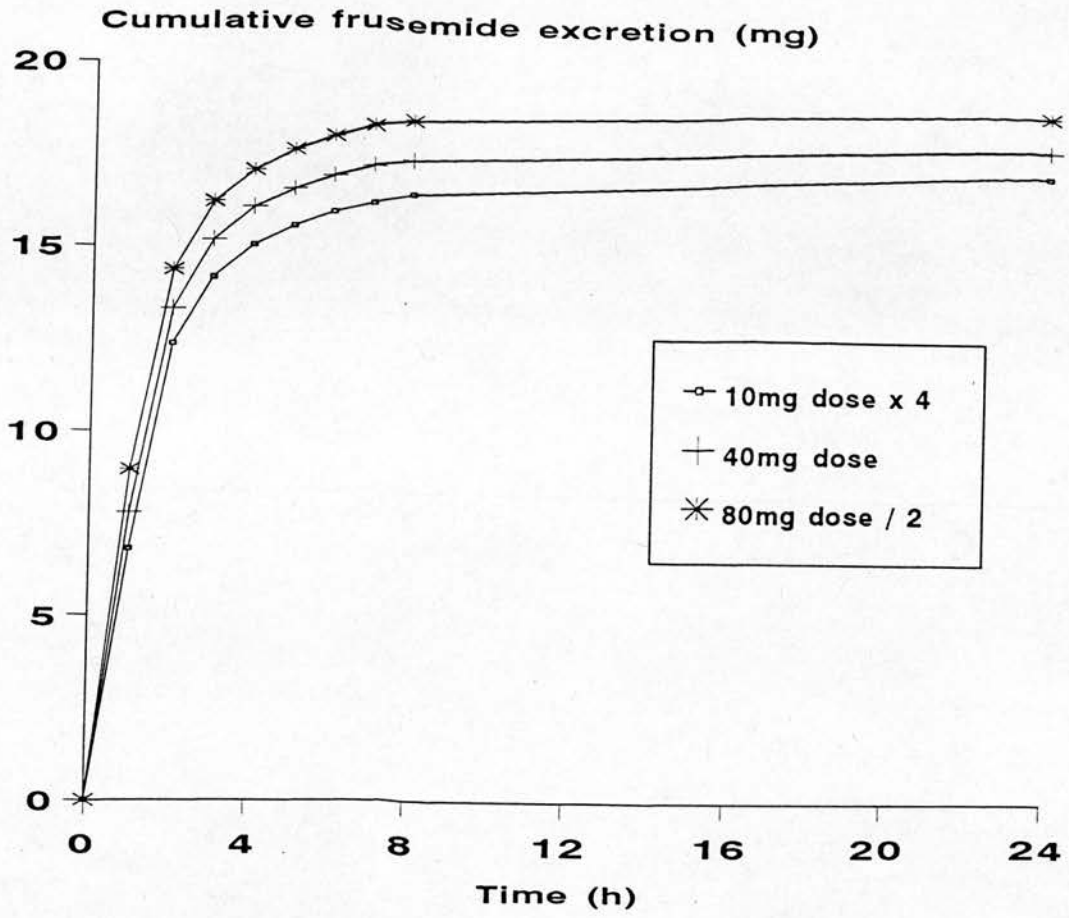

Figure 7.4. Normalised frusemide excretion following administration of 10, 40 and 80 mg to 8 healthy volunteers.

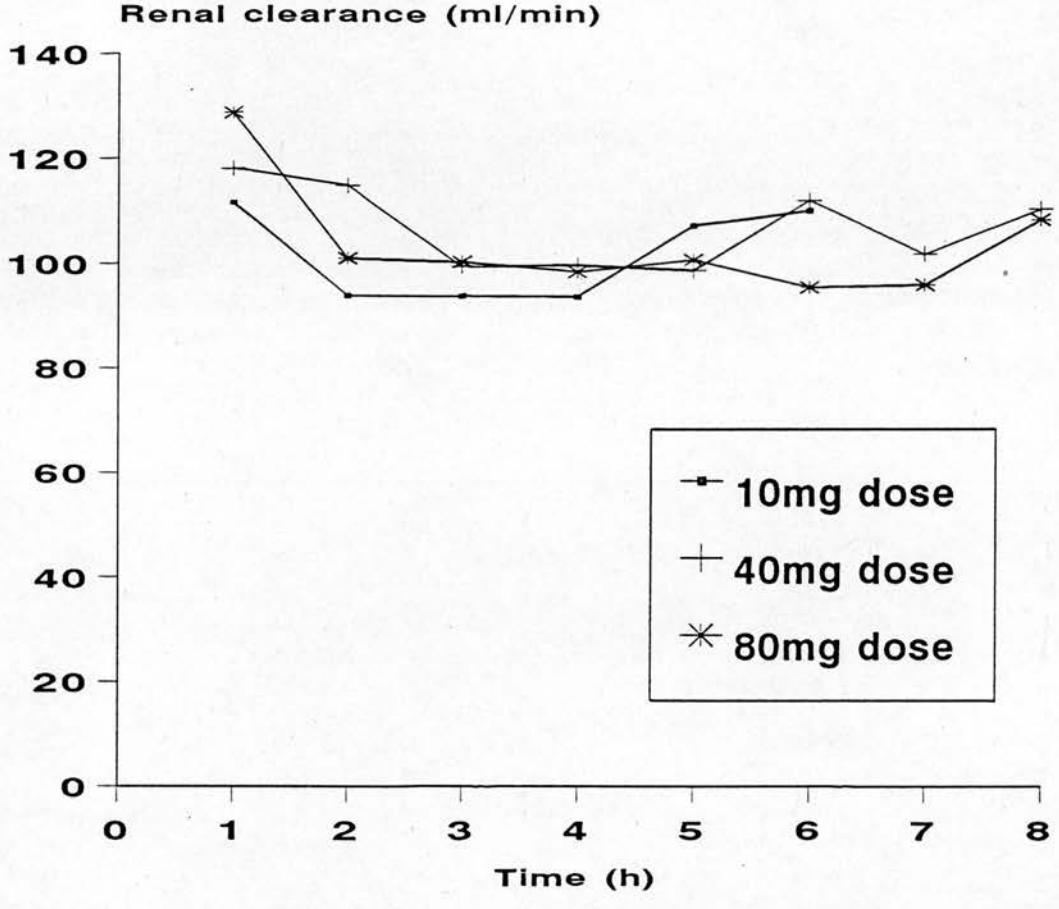

Figure 7.5. Mean hourly renal clearances following administration of 10, 40 and 80 mg to 8 healthy volunteers.

Section 7.4. Discussion

Dose-dependent kinetics are seen within the therapeutic dose range for drugs such as phenytoin and salicylic acid (Rowland and Tozer, 1980). The pharmacokinetic behaviour of two antibiotics, cefadroxil and amoxycillin, have also recently been studied, since these drugs may be subject to active absorption from the intestine (Sánchez-Picó *et al.* 1989) and active renal tubular secretion (La Rosa *et al.* 1982). Garrigues *et al* (1991) showed that the absorption and elimination kinetics of cefadroxil were non-linear after oral administration in man. Normalised peak plasma concentrations and areas under the plasma concentration time curves decreased significantly with increasing dose. Its clearance was also shown to be concentration-dependent. Chulavatnatol & Charles (1994) using amoxycillin showed that the fraction of the dose excreted unchanged decreased from 0.5 after 97 mg to 0.23 after 3103 mg in healthy volunteers. Sjövall *et al* (1992) administered increasing doses of amoxycillin to patients with ileostomy. In the urine 70 % was recovered at the lowest dose compared with 23 % at the highest dose. Cook *et al* (1988) has suggested the possibility that the absorption of the other commonly used diuretic bumetanide may be dose-dependent. They obtained 81 % bioavailability after a 3 mg dose. This value was less than the 89 % mean bioavailability reported by Marcantonio *et al* (1982), who administered 1 mg and greater than the 66 % bioavailability reported by Lau *et al* (1986) who gave a 5 mg dose to healthy subjects.

In this study however there was no evidence for a significant dose-dependent effect on frusemide absorption or disposition over the dosage range studied. Normalised 10 and

80 mg frusemide plasma concentrations and amounts excreted were not significantly different compared to the 40 mg dose. Renal clearance of frusemide also remained constant which is indicative of linear pharmacokinetics i.e. no evidence of concentration dependent (dose-dependent) renal clearances of frusemide.

It is possible that a dose effect may be seen with a wider dose range, however it would not be possible to study this in healthy volunteers due to ethical considerations. However its absorption kinetics are clearly linear over the range 10 - 80 mg, which includes the most commonly used doses in clinical practice.

CHAPTER 8

PENICILLIN AND FRUSEMIDE INTERACTION IN MAN

Section 8.1. Introduction

It is not uncommon for patients to take several drugs simultaneously. Pharmacokinetic drug interactions can therefore arise, where one drug affects the absorption, distribution, metabolism or excretion of another.

Being a weak acid, with extensive protein binding, the majority of frusemide is thought to reach its site of action in the kidney via the nonspecific organic acid secretory pathway of the proximal tubule (Bowman, 1975; Cutler & Blair, 1979; Hook & Williamson, 1965). Many other acidic drugs are secreted by the same transport mechanism and competition for renal secretion can occur. Both probenecid (Brater, 1978; Chennavasin *et al.* 1979; Homeida *et al.* 1977; Honari *et al.* 1977; Odlind and Beermann, 1980) and indomethacin (Data *et al.* 1978; Smith *et al.* 1979) have been shown to compete with frusemide for secretion, resulting in decreased renal clearance of the drug.

Benzylpenicillin (penicillin G) has a similar half-life to frusemide and is removed by proximal tubular secretion (Kampmann *et al.* 1972). It is therefore possible that benzylpenicillin and frusemide may compete for renal secretion, however no studies have been carried out to determine this possible interaction. If benzylpenicillin alters the renal clearance and excretion of frusemide, changes in the duration and magnitude of its diuretic action may result. Benzylpenicillin is still widely used for the treatment of infections caused by susceptible microorganisms (Wise, 1982; Ball, 1982).

The purpose of this study was to determine if frusemide and benzylpenicillin compete for renal secretion and secondly to determine if any interaction alters the natriuretic and

diuretic action of frusemide.

Section 8.2. Methods

Subjects

Eight healthy male volunteers aged 21 - 40 years (average 32 ± 7 yrs) and weighing 54 to 95 kg (average 77 ± 14 kg) were studied. They were healthy according to history, clinical examination and haematological and biochemical tests. Volunteers were excluded if they were allergic to penicillin or if they had any history of asthma or eczema. All volunteers were asked to avoid any other medication for 1 week prior to and throughout the study. For 2 days prior to each study period they were asked to avoid excessive intake of dietary salt. Each volunteer was given a diet sheet for guidance (see Appendix 3). Volunteers were also asked to avoid alcohol for 24 h before each study day, avoid caffeine containing drinks (coffee, tea, cola etc.) from 18.00 h and fast from 20.00 h the evening before each study day. The study was approved by the Lothian Health Board Healthy Volunteer Studies Ethics of Medical Research Sub-Committee and each volunteer gave informed written consent before taking part.

Procedure

Each volunteer was studied on 3 occasions at least one week apart, using a randomised design. On each occasion the volunteer drank 200 ml of water at home at 07.00 h. At approximately 08.00 h they attended the Clinical Pharmacology Unit, emptied their bladders and drank a further 200 ml of water. An intravenous cannula (Venflon 2, 18G) was then placed into a vein in each forearm, one for the administration of drugs and one

for blood sampling. The volunteers remained recumbent for 1 h. The volunteers then emptied their bladders again and received one of the following:

(1) 40 mg intravenous frusemide (10 mg/ml "Lasix" injection) - 4 ml frusemide injection + 46 ml 0.9 % sodium chloride solution, infused at a constant rate over 10 min.

(2) 2.4 g intravenous penicillin (Benzylpenicillin sodium) dissolved in 45 ml of water followed by 40mg intravenous frusemide (4 ml frusemide injection + 1 ml 0.9 % sodium chloride). Both were infused into the same arm according to the schedule shown in Fig. 8.1.

(3) Placebo (No drug control) - 50 ml of 0.9 % sodium chloride solution infused at a constant rate over 10 min.

The vials of frusemide and penicillin used in the study contained different concentrations of sodium chloride. The solutions were therefore made up as described above in order to ensure that the same amount of sodium (7.46 mmol) was administered during each infusion. A total volume of 50 ml was infused on each occasion.

On all 3 occasions, the volunteers drank 150 ml of water every half-hour for 6 h. A light lunch was provided at the end of each 6 h study period.

Venous blood samples (5 ml) were taken from the opposite arm just before frusemide and at 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 180, 240, 300, 360 min after frusemide administration. Samples were collected in lithium heparin tubes and centrifuged at 3000 rpm for 15 min. Plasma was stored at -20^{0}.

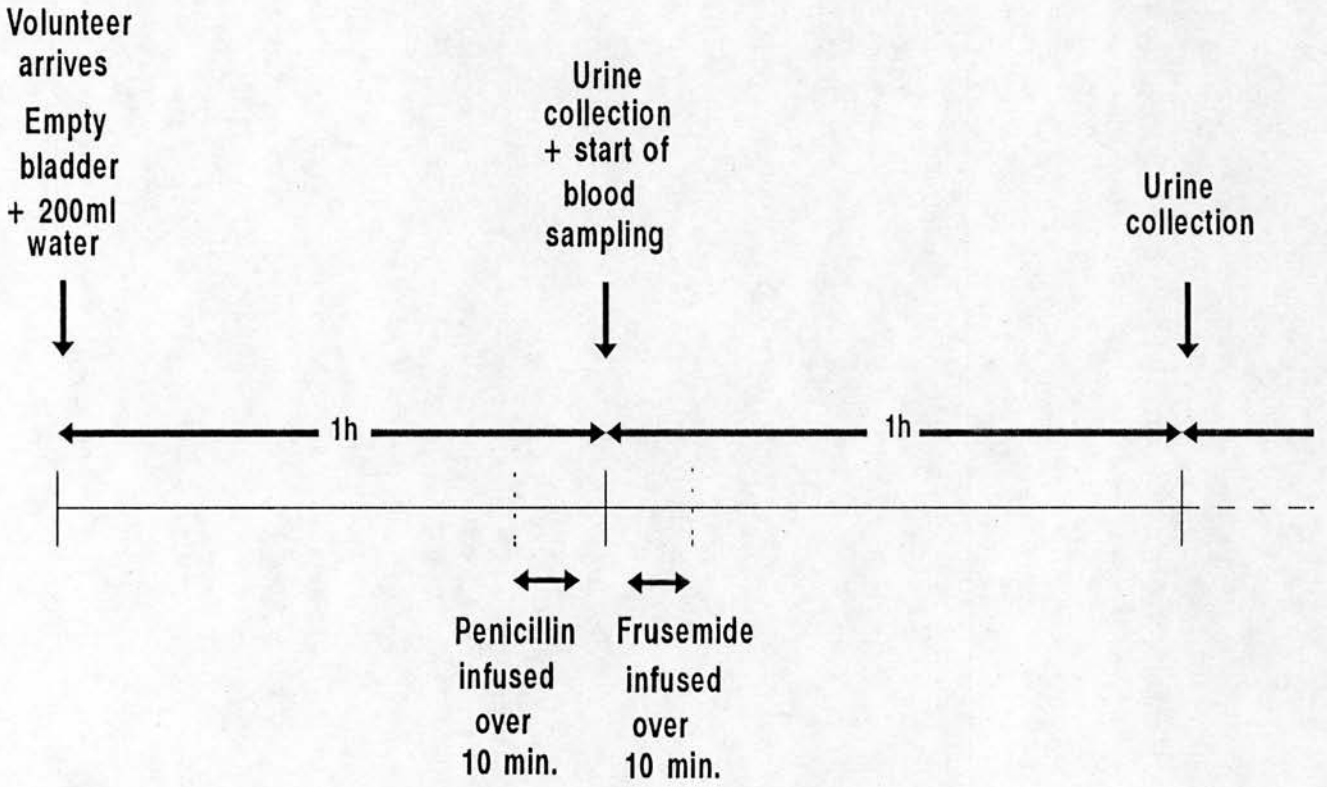

Figure 8.1. Time schedule for penicillin and frusemide administration

On all three occasions urine was collected at 0, 1, 2, 3, 4, 5 and 6 h. Volunteers also collected urine from 6 - 24 h at home. Urine volumes were recorded and aliquots stored at -20^0 until analysis.

Analysis of samples

Frusemide concentrations in plasma and urine were measured by high performance liquid chromatography and urinary sodium and potassium concentrations by ion specific electrodes (Chapter 2).

Pharmacokinetic analysis

Following the intravenous infusion of frusemide plasma concentration versus time curves were described by a 2 compartment model according to Equation 2.14. Data were analysed by computer fitting using "Siphar". Total clearance and renal clearance of frusemide were calculated using Equations 2.8 and 2.9. Non renal clearance was defined as the difference between total and renal clearance.

Statistics

All data are expressed as means ± standard deviations (s.d.). Statistical significant differences were determined using the Students t-test and Analysis of variance. P values of <0.05 were accepted as significant.

Section 8.3. Results

Pharmacokinetics

The pharmacokinetics of frusemide administered alone (F) and with penicillin (F+P) are presented in Table 8.1. Total plasma clearance of frusemide was significantly reduced when administered with penicillin (160.1 ± 30.7 for F and 132.6 ±22.4 ml/min for F+P, $P<0.05$). The change in total clearance was due to a reduction in mean frusemide renal clearance with penicillin (103.0 ± 15.9 for F versus 83.6 ±17.4 ml/min for F+P, $P<0.05$). The reduction in renal clearance was however not persistent throughout the study. Renal clearance values were only significantly reduced by penicillin over the first 2 h compared to frusemide alone (Fig 8.2). The non renal clearance did not differ significantly between treatments.

The reduced plasma clearance of frusemide administered with penicillin resulted in slightly higher plasma concentrations of the diuretic at all time points, except over the first 10 min (Fig 8.3). However, no significant difference was found in mean $AUC_{0\text{-}6h}$ (4.36 ± 1.05 for frusemide alone and 5.16 ± 0.79 mg.h/l for frusemide and penicillin).

The urinary excretion rates of frusemide administered alone and with penicillin are shown in Fig 8.4. A reduction in the urinary excretion rate of frusemide was found over the first hour following administration with penicillin. For the remainder of the study frusemide excretion rates were not significantly different. No significant difference in total urinary recovery of frusemide over 24 h was found (Fig. 8.5.)

Mean plasma half-life was not significantly different following administration of

Table 8.1. Pharmacokinetic parameters of intravenous frusemide administered alone (F) or with intravenous penicllin (F + P) in 8 healthy subjects. Values are means ± s.d.

Subject	Half-life (h)		Vd (L/kg)		CL_T (ml/min)		CL_R (ml/min)		CL_{NR} (ml/min)	
	F	F+P	F	F+P	F	F+P	F	F+P	F	F+P
1	1.10	0.98	0.22	0.16	122.8	101.5	89.1	62.2	33.7	39.3
2	1.17	0.97	0.22	0.14	177.3	128.7	101.3	92.5	76.0	36.2
3	0.90	1.14	0.08	0.16	180.6	132.3	124.6	85.4	56.1	46.9
4	1.40	1.16	0.24	0.20	191.6	182.2	122.4	123.0	69.2	59.2
5	1.02	1.02	0.16	0.12	177.3	123.7	104.4	83.2	72.9	40.5
6	0.92	1.94	0.11	0.27	100.4	118.4	70.1	79.4	30.3	39.0
7	0.90	0.90	0.17	0.16	149.5	148.5	97.2	68.9	52.3	79.6
8	0.86	1.49	0.22	0.26	181.6	126.0	114.0	73.9	67.6	52.1
Mean	1.04	1.20	0.18	0.18	160.1	132.6	103.0	83.6	57.3	49.1
S.D.	0.18	0.35	0.06	0.05	30.7	22.4	15.9	17.4	17.5	14.6
P	NS		NS		<0.05		<0.05		NS	

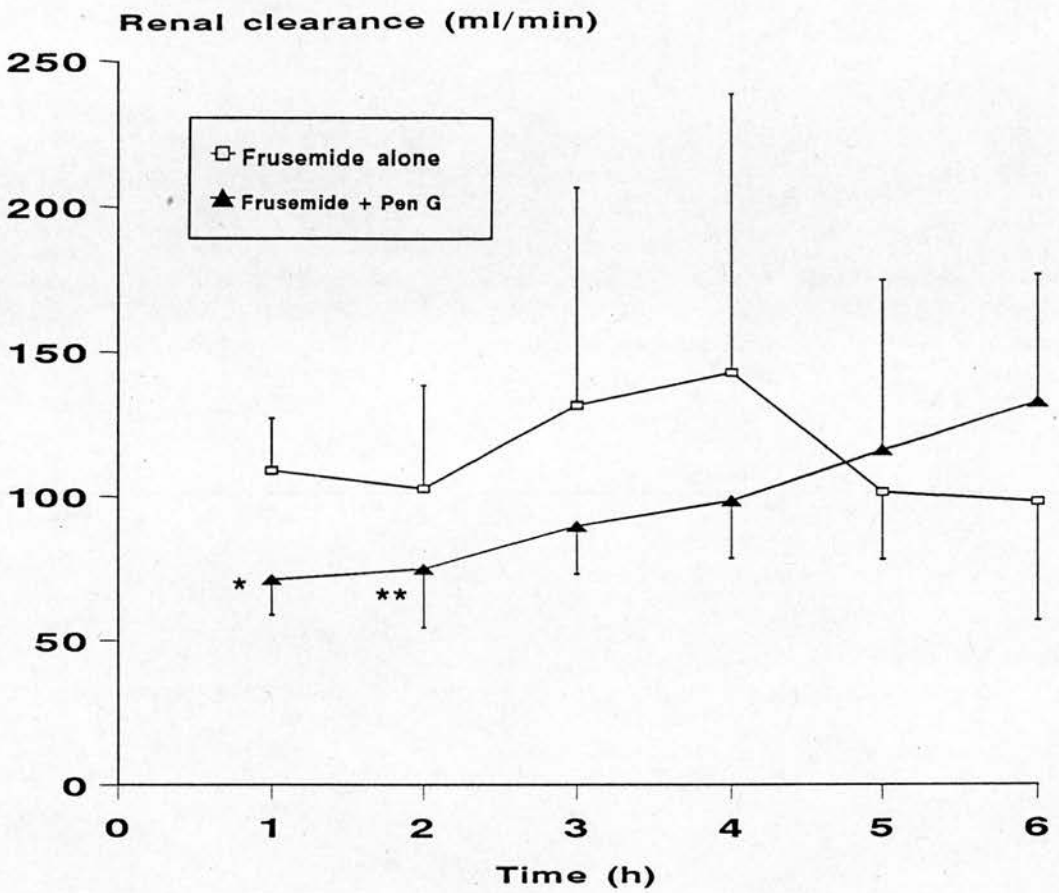

Figure 8.2. Effect of penicillin on renal clearance of frusemide over time. *P<0.01, compared to frusemide alone: **P<0.05, compared to frusemide alone. Bars = ± s.d.

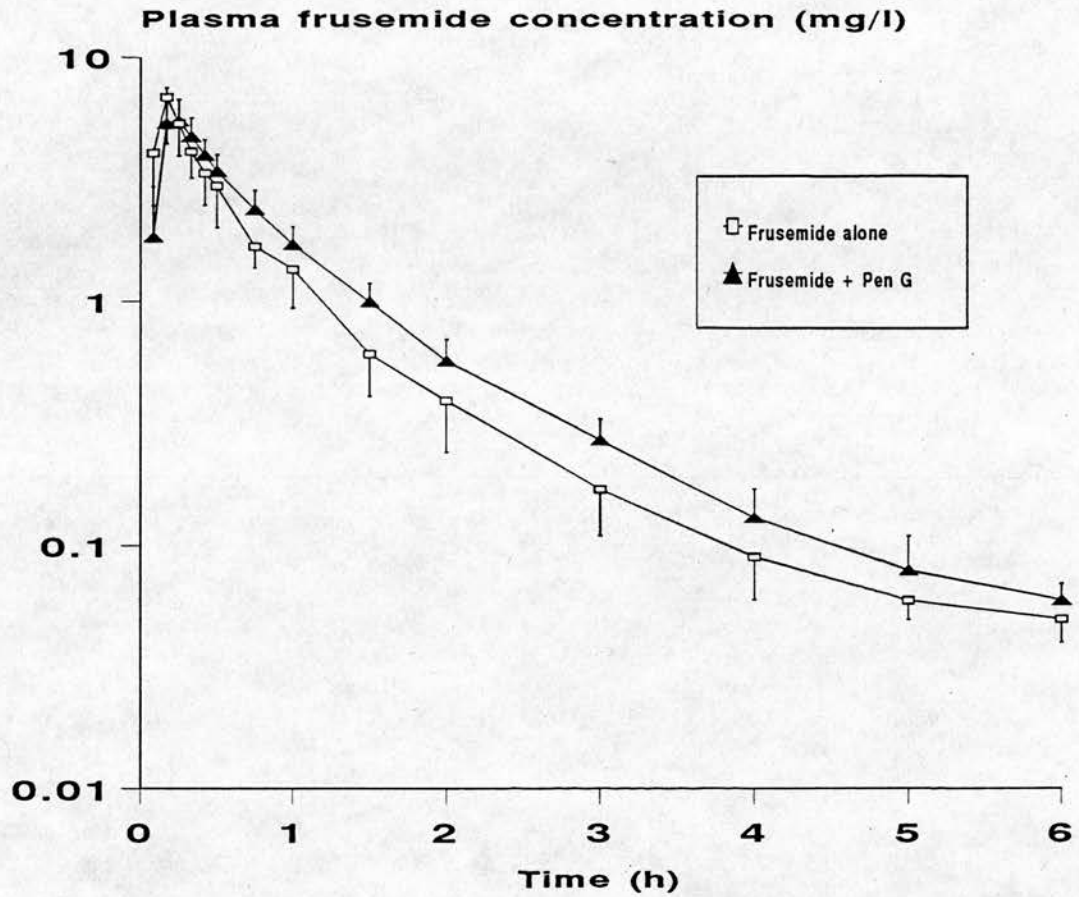

Figure 8.3. Mean plasma concentrations following administration of frusemide alone and with penicillin in 8 healthy volunteers. Bars = ± s.d.

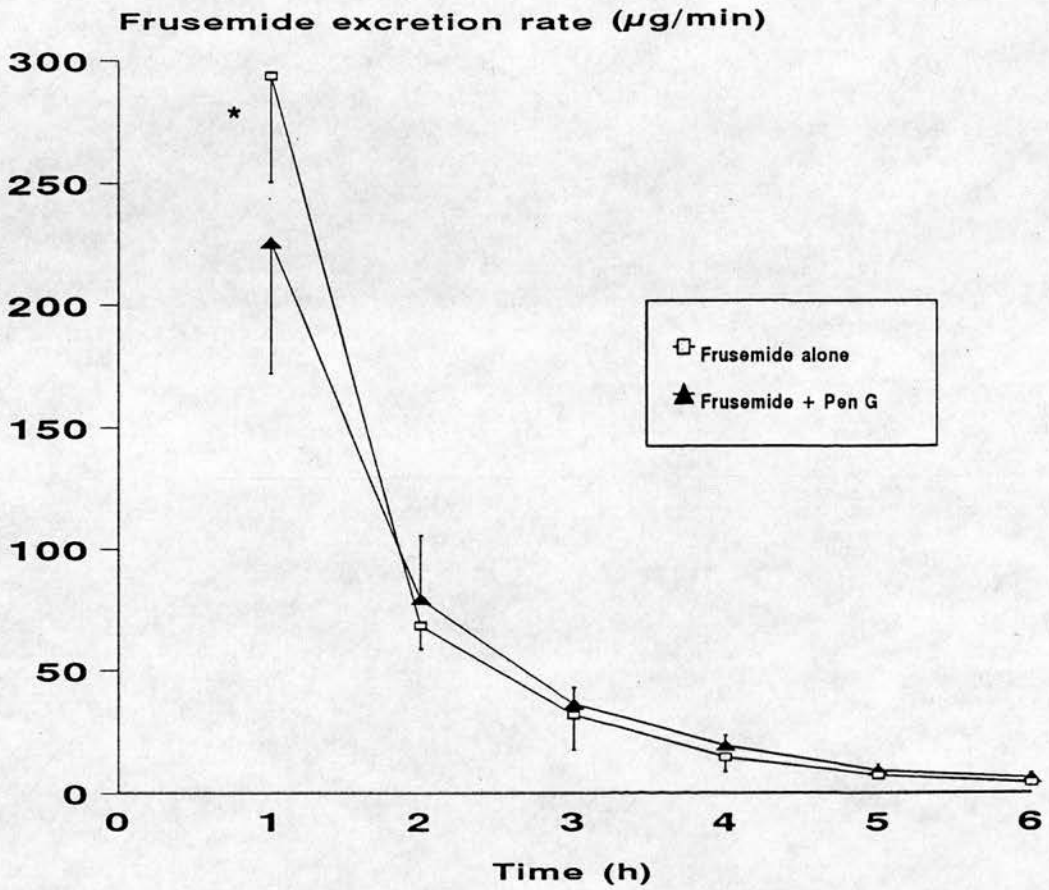

Figure 8.4. Urinary excretion rate of frusemide administered alone and with penicillin in 8 healthy volunteers. *P<0.05, compared to frusemide + penicillin.

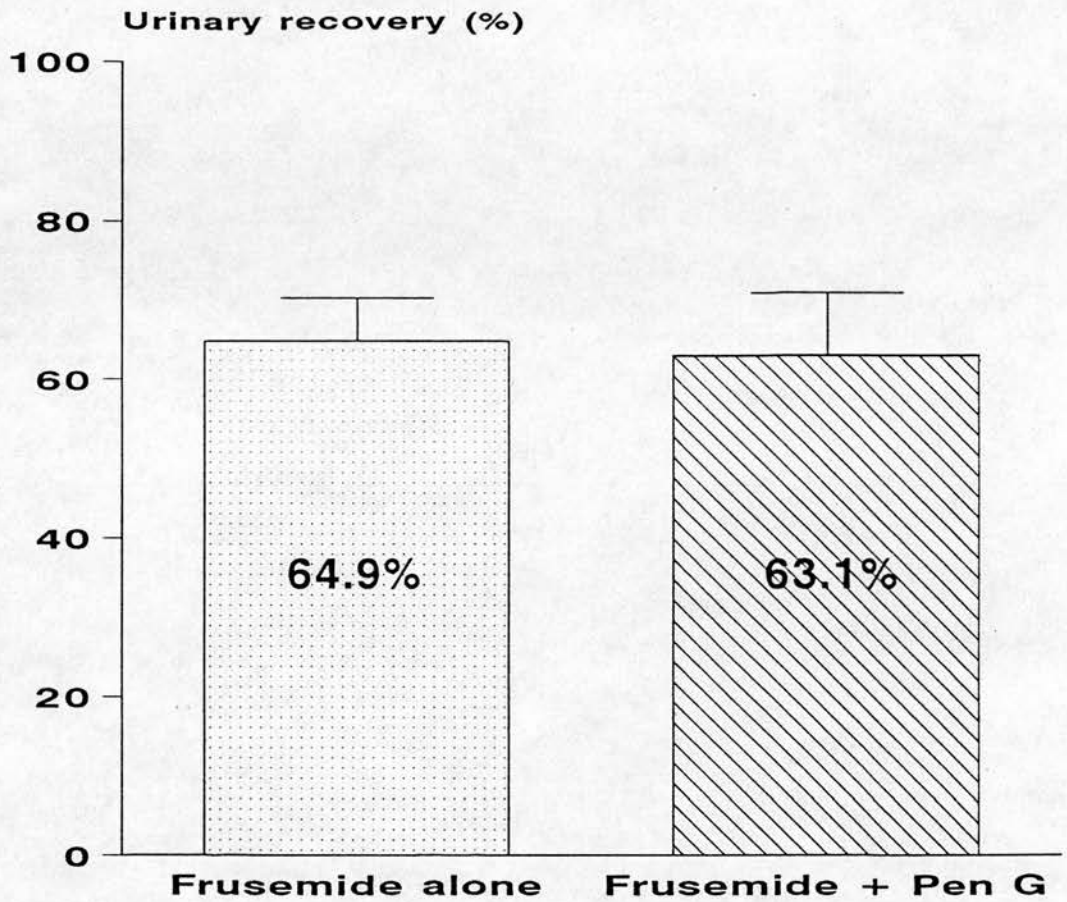

Figure 8.5. Total urinary recovery of frusemide over 24h (% of administered dose) following administration of frusemide alone and with penicillin in 8 healthy volunteers.

frusemide alone or with penicillin. The volume of distribution (Vd) was 0.18 L/kg; no change occurred after penicillin.

Response to frusemide

The time courses of frusemide induced sodium, water and potassium excretion are shown in Figs. 8.6, 8.7 and 8.8. The effect of penicillin on total sodium, urine and potassium output is shown in Figs. 8.9, 8.10 and 8.11.

Sodium excretion

Hourly sodium excretion rates remained constant following placebo (Fig 8.6). Sodium excretion rates following intravenous frusemide were not altered by penicillin. Following both frusemide alone and frusemide with penicillin, sodium excretion rates peaked at 1 h and declined thereafter. 5 and 6 h after frusemide administration sodium excretion rates were lower compared to those found during the placebo. Total sodium output over 6 h were not significantly different following administration of frusemide alone and frusemide with penicillin. Both were significantly higher compared with placebo (Fig. 8.9).

Urine volume

During the placebo study urine flow rate increased slightly over the first 2 h but remained constant over the next 4 h (Fig. 8.7). Following administration of frusemide alone and with penicillin, urine flow rates were almost identical at all time points. Flow rates peaked at 1 h, followed by a decrease during subsequent hours. Urine flow rates were

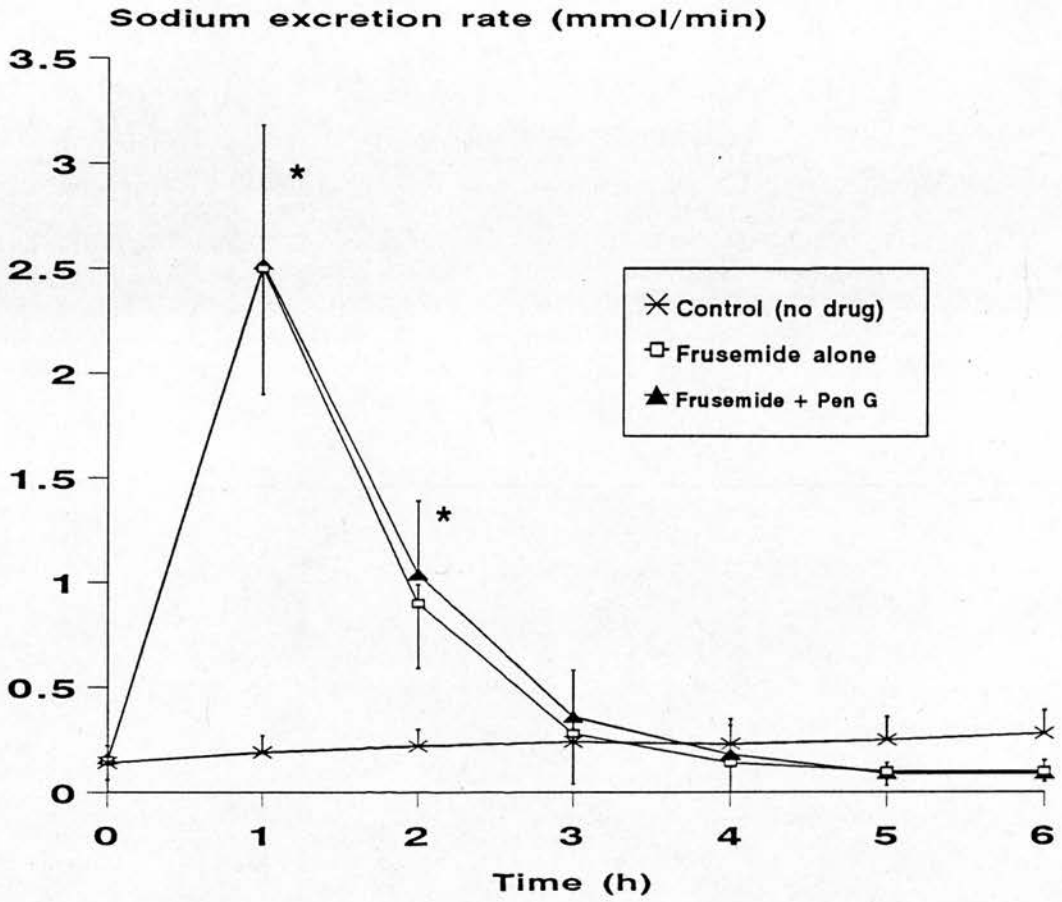

Figure 8.6. Sodium excretion rates following administration of placebo, frusemide alone and frusemide with penicillin in 8 healthy volunteers. *P<0.001, compared to control.

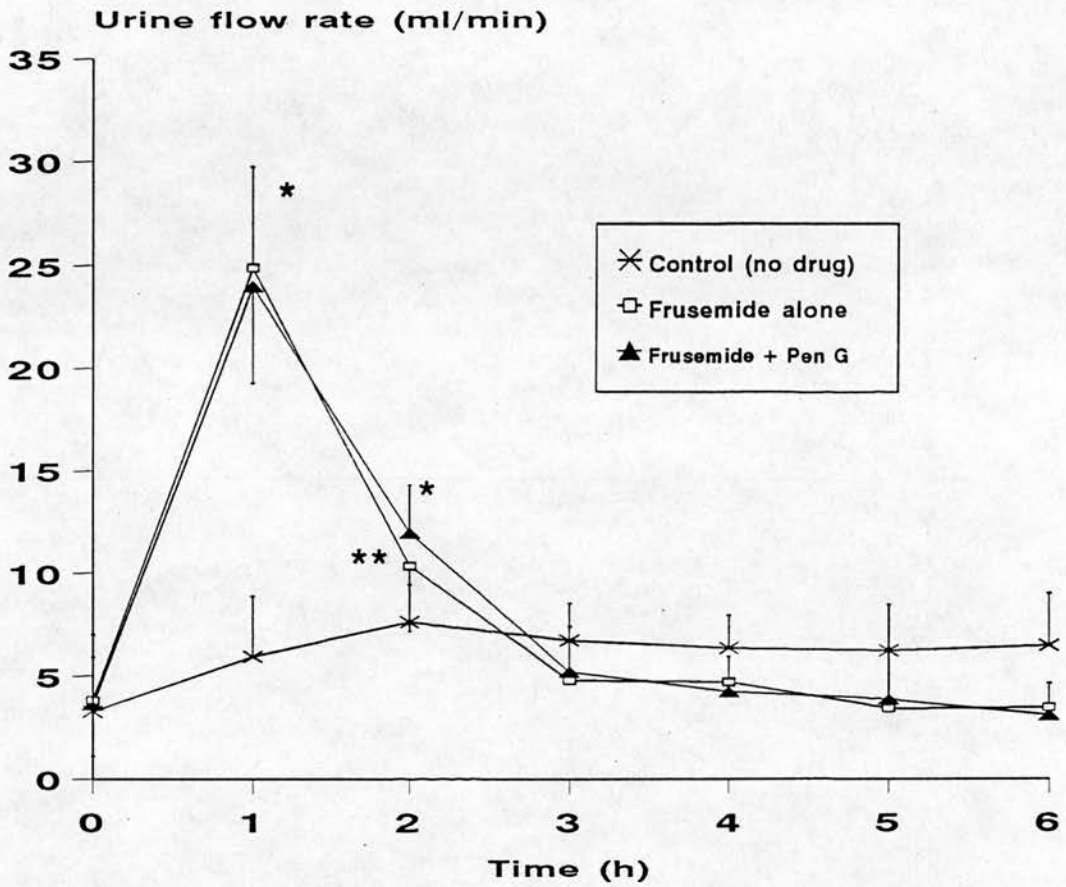

Figure 8.7 Urine flow rates following administration of placebo, frusemide alone and frusemide with penicillin in 8 healthy volunteers. *P<0.01, compared to control: **P<0.05, compared to control

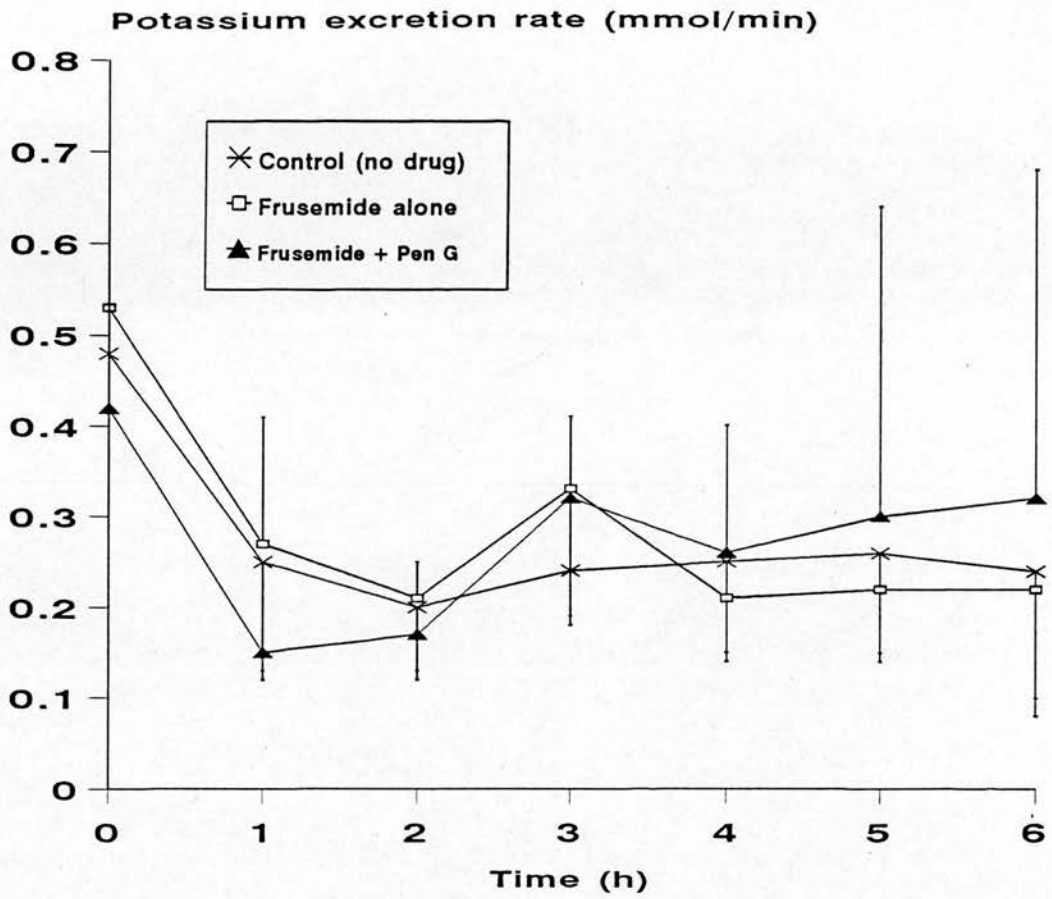

Figure 8.8 Potassium excretion rates following administration of placebo, frusemide alone and frusemide with penicillin in 8 healthy volunteers.

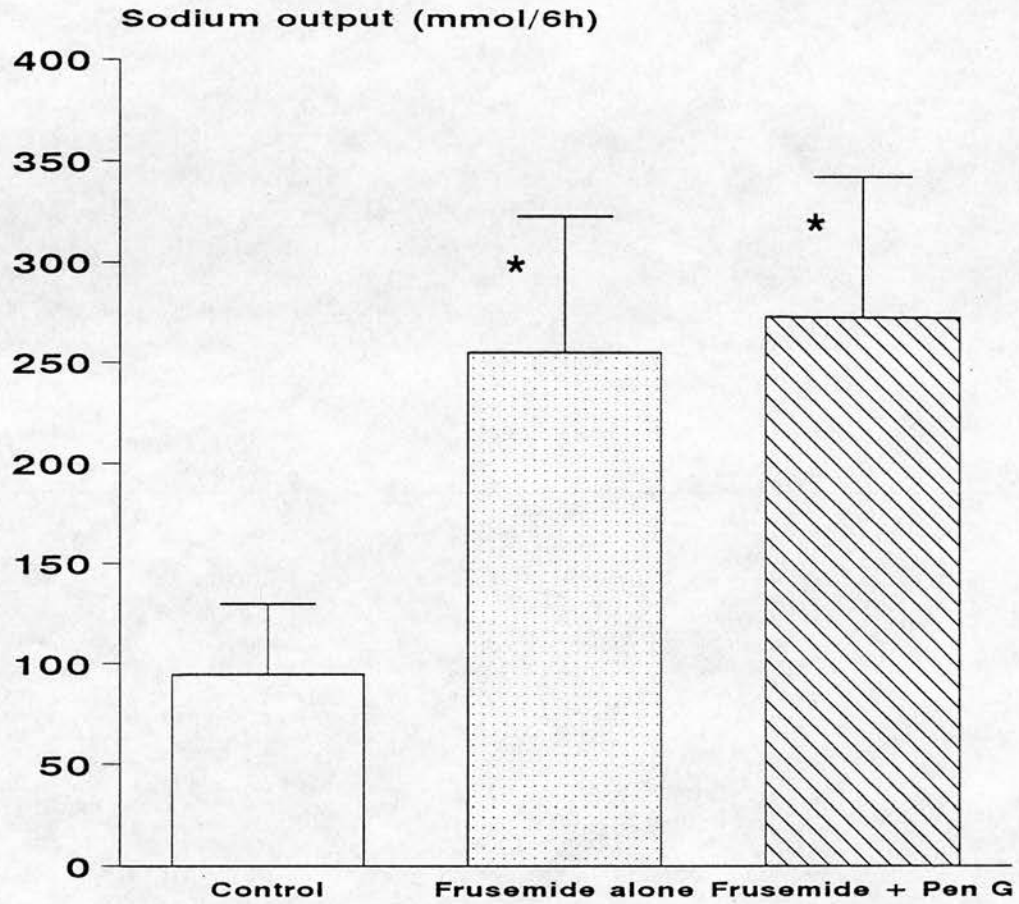

Figure 8.9. Total sodium output over 6h following administration of placebo, frusemide alone and frusemide with penicillin in 8 healthy volunteers. *P<0.001, compared to control.

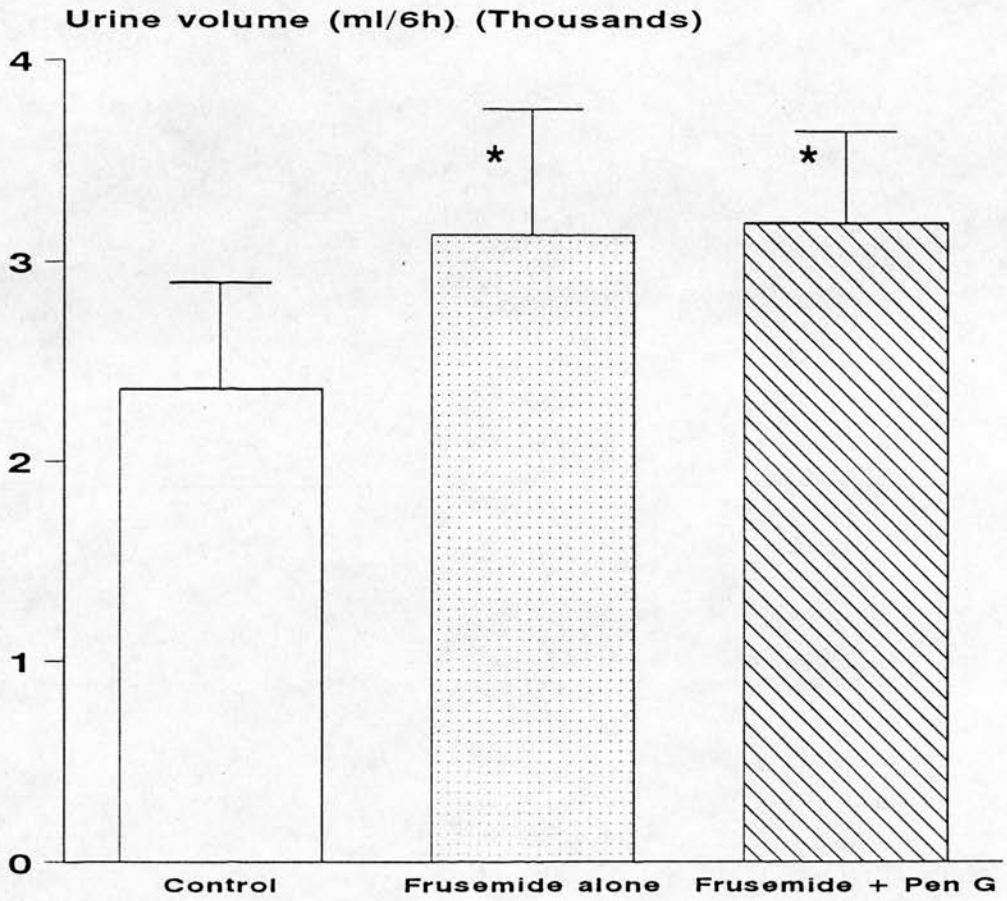

Figure 8.10. Total urine volume over 6h following administration of placebo, frusemide alone and frusemide with penicillin in 8 healthy volunteers. *P<0.01, compared to control.

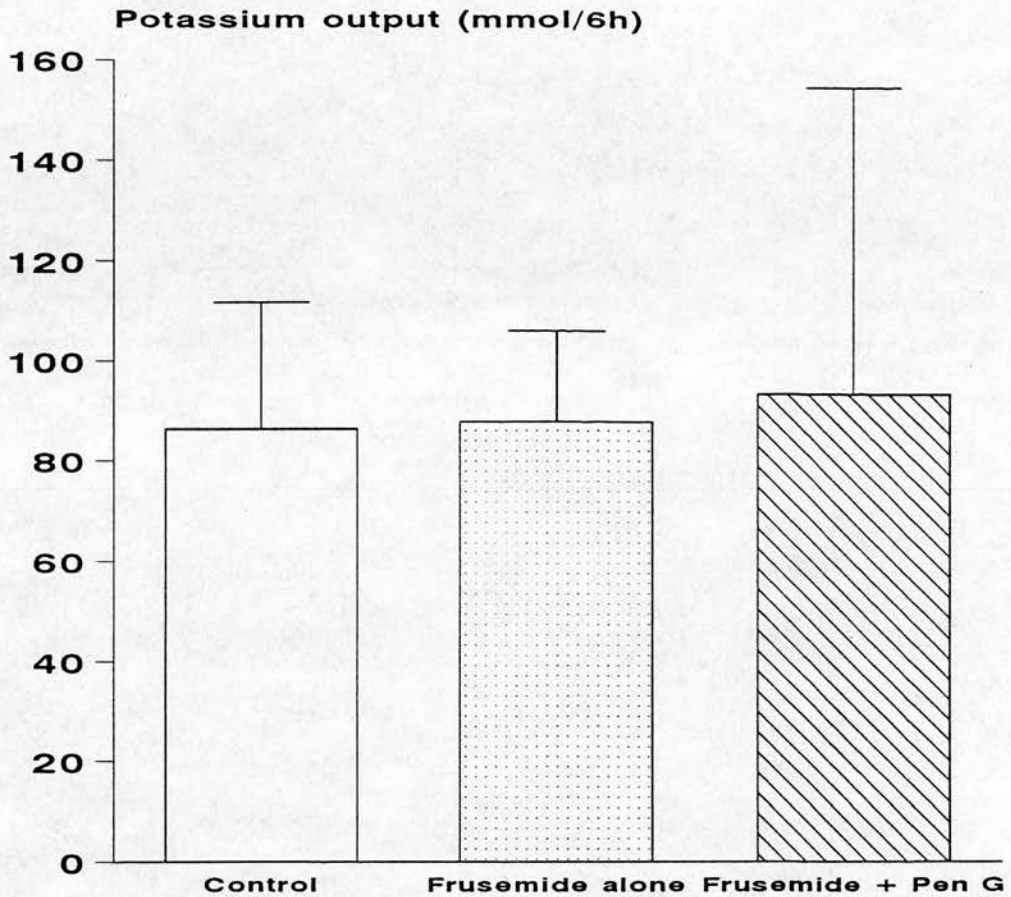

Figure 8.11. Total potassium output over 6h following administration of placebo, frusemide alone and frusemide with penicillin in 8 healthy volunteers.

lower compared to those found during the placebo study from 3 h onwards. Total urine volumes over 6 h were not significantly different following administration of frusemide alone and with penicillin (Fig. 8.10).

Potassium excretion

Potassium excretion rates were found not to be significantly different at any time point over the 6 h study period following placebo, intravenous frusemide and intravenous frusemide with penicillin. On all 3 occasions potassium excretion rates decreased from baseline values over the first hour. Excretion rates did not show any increase following frusemide administration, except at 3 h. Total potassium output over 6 h for placebo, frusemide alone and frusemide with penicillin were not significantly different (Fig. 8.11).

Section 8.4. Discussion

Frusemide is a weak acid which is primarily excreted by proximal tubular secretion. The substantial protein binding of the drug limits glomerular filtration (Cutler *et al.* 1974: Cutler & Blair, 1979; Forrey *et al.* 1974). It might be expected that other organic acids using the same transport system could inhibit frusemide secretion, reduce the amount in the tubular lumen and hence reduce its diuretic effect. Potential drug-drug interactions at this site have previously been evaluated using drugs such as probenecid and pentopril.

Probenecid is a known competitive inhibitor of secreted organic acids and was at one time used in the hyperuricemia associated with frusemide therapy (Weiner *et al.* 1960). In animals, coadministration of probenecid reduced frusemide secretion and attenuated its natriuretic affect (Friedman *et al.* 1977; Hook & Williamson, 1965). However

studies in healthy volunteers have resulted in contradictory results (Chennavasin *et al.* 1979; Homeida *et al.* 1977; Honari *et al.* 1977; Odlind and Beermann, 1979; Sommers *et al.* 1991). All the studies demonstrated a marked reduction in the renal clearance of frusemide by probenecid. In some cases probenecid also decreased the nonrenal clearance of frusemide (Chennavasin *et al.* 1979) or its volume of distribution (Homeida *et al.* 1977). Odlind and Beermann (1979) and Honari *et al* (1977) further demonstrated that the reduction in renal clearance and excretion of frusemide caused by probenecid attenuated the natriuretic effect. In contrast several other studies reported that sodium excretion remained unchanged (Sommers *et al.* 1991; Smith *et al.* 1980) or even showed an increase (Chennavasin *et al.* 1979; Brater, 1978) after probenecid, despite the substantial reduction in the renal clearance and excretion of frusemide. Chennavasin *et al* (1979) suggested that accumulation of frusemide in plasma from concomitant effects of probenecid on its nonrenal clearance allowed more frusemide to appear in the urine at later times, increasing response.

Tubular secretion also contributes significantly to the renal elimination of pentopril, an angiotensin-converting enzyme inhibitor. The pharmacokinetic interaction of oral frusemide and pentopril has been studied in 12 healthy volunteers (Rakhit *et al.* 1987). Pentopril induced significant changes in the disposition of frusemide. Pentopril decreased the renal clearance of frusemide by 54 % and the fraction excreted unchanged by 55 %. This was compensated for by an increase in the metabolism of frusemide by glucuronidation. However, in spite of the decreased renal clearance and urinary excretion rate of frusemide, urinary output and sodium excretion remained almost unchanged. One possible explanation was that total frusemide (unchanged and

glucuronide) might contribute to diuresis and natriuresis rather than unchanged frusemide alone. These studies show that drug interactions with frusemide at this site are complex.

In the present study intravenous benzylpenicillin was found to reduce the renal clearance of frusemide presumably by competing with frusemide for proximal tubular secretion. The effect of benzylpenicillin on organic acid transport was however short-lived. Urinary excretion of frusemide was only reduced by benzylpenicillin over the first hour of the study, possibly because of more rapid elimination of penicillin. Frusemide non renal clearance, volume of distribution and elimination half-life were not altered. The interaction also had no effect on the total urinary recovery of frusemide or on the time course and magnitude of natriuresis and diuresis.

In conclusion, the large intravenous dose of benzylpenicillin used in this study did not affect the renal handling of frusemide to any great extent. In clinical practice, benzylpenicillin is also usually administered intravenously because the absorption from oral dosage forms is poor (Martindale, 1993). It is therefore unlikely that coadministration of these two drugs clinically would alter the diuretic and natriuretic response to frusemide.

CHAPTER 9

EFFICACY OF SINGLE VERSUS REPEATED DIVIDED DOSES OF FRUSEMIDE

Section 9.1. Introduction

Frusemide is usually administered either orally or as an intravenous bolus. In 1982 Kaojarern *et al.* demonstrated in healthy volunteers that virtually the same natriuretic response was obtained whether the same dose of frusemide was administered intravenously or orally, despite half as much unchanged drug being excreted after the oral dose. They suggested that the time course of delivery of frusemide to the active site was an independent determinant of overall response. Since then several reports in healthy volunteers and patients have also shown that continuous intravenous infusion of loop diuretics leads to increased diuretic and natriuretic effects compared to bolus administration (Lahav *et al.* 1992; Lawson *et al.* 1978; Meyel *et al.* 1992; Rudy *et al.* 1991). One explanation is that the infusion may provide a more efficient rate of drug delivery into the urine (Alvan *et al.* 1990; Meyel *et al.* 1992). In addition, the administration of large single doses of diuretics can lead to the development of acute tolerance. This is thought to be caused by compensatory renal sodium retention because it occurs in response to excessive sodium excretion and is correlated with both the rate and magnitude of natriuresis (Andreasen *et al.* 1989; Cook and Smith, 1987; Hammarlund *et al.* 1985; Zhu & Koizum, 1987; Wilcox *et al.* 1983). Plasma concentrations of renin and aldosterone increase consistently after frusemide (Mackay *et al.* 1984; Riley *et al.* 1985; Sjöstrom *et al.* 1988b; Wilcox *et al.* 1983) and it has been proposed that the renin-angiotensin-aldosterone system (Figure 9.1) may play a role in mediating the development of acute tolerance (Sjöstrom *et al.* 1988b). One of the effects of angiotensin II is to increase the synthesis and release of aldosterone which in turn increases sodium reabsorption. The development of this type of tolerance may

have been minimised by constant low dose infusion.

A prolonged low level response to frusemide may also be obtained if small repeated oral doses were given at regular intervals. If this led to an increased total response an oral sustained release preparation of frusemide may be beneficial To test this hypothesis we compared the kinetic and the dynamic effects of a single oral dose of frusemide with an equivalent oral dose administered at hourly intervals over 8 h in healthy volunteers. Plasma renin activity (PRA) and aldosterone plasma concentrations were also measured to determine any differences in patterns of response during single and repeated frusemide administration.

Section 9.2. Methods

Subjects

Eight healthy male volunteers, aged 25 - 36 years (30 ± 5 yrs) and weighing 55 - 96 kg (77.3 ± 12.6 kg) participated in the study. They were healthy according to medical history, clinical examination and haematological and biochemical tests. Each volunteer gave informed written consent to the study, which was approved by the Lothian Health Board Healthy Volunteer Studies Ethics of Medical Research Sub-Committee. The volunteers were asked to observe the following whilst taking part in the study:

(1) avoid any other medication for 1 week prior to and throughout the study
(2) avoid excessive intake of dietary salt for 2 days prior to each study day. Each volunteer was given a diet sheet for guidance (Appendix 3).
(3) collect urine for 24 h at home before each study period. This collection was completed on arrival in the Clinical Pharmacology Unit and was used to assess sodium and potassium excretion and thus approximate dietary intake of sodium and potassium.
(4) avoid alcohol for 24 h before each study

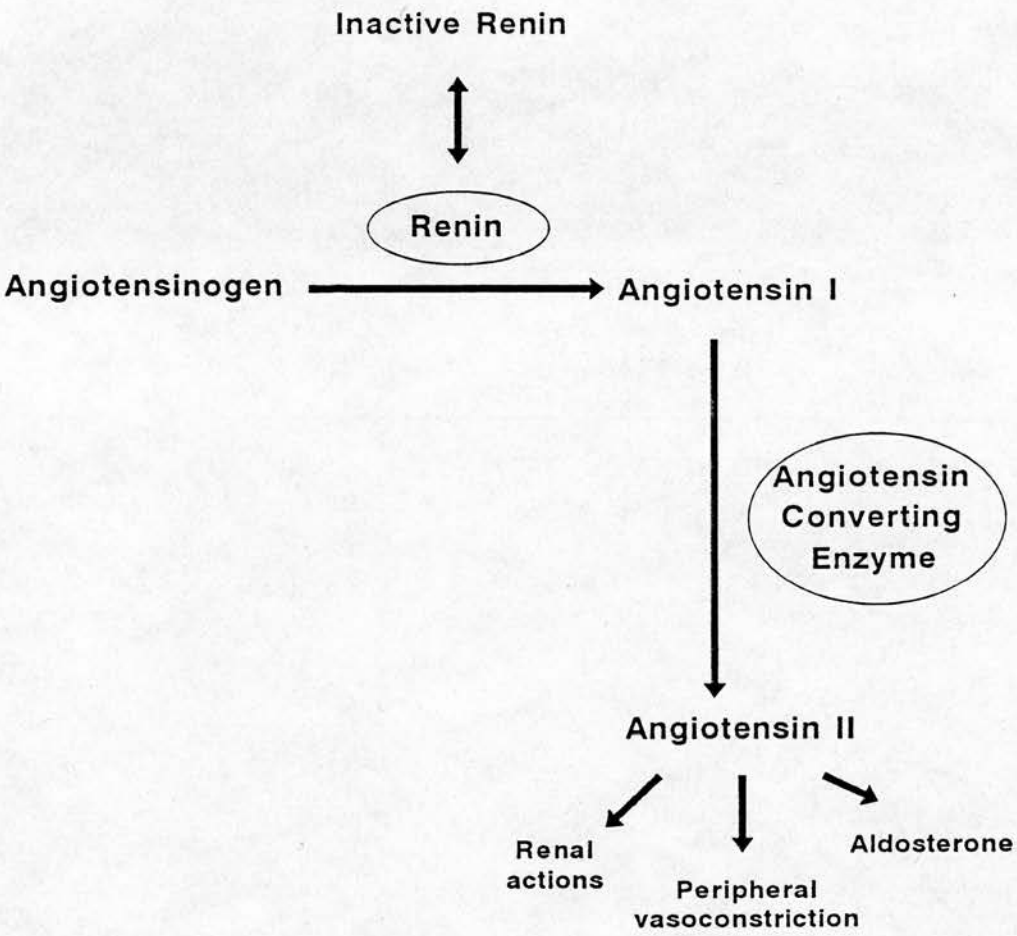

Figure 9.1. Renin-angiotensin-aldosterone system in man.

(5) avoid caffeine (coffee, tea, cola) from 18.00 h the evening before each study day
(6) fast from 22.00 h the evening before each study day

Procedure

A randomised, cross-over, single blind design was used. Each volunteer was studied on 2 separate occasions at least one week apart. At approximately 07.00 h the volunteers drank 200 ml of water at home. One hour later they attended the Clinical Pharmacology Unit. On arrival the volunteers emptied their bladders completely. This completed their control 24 h urine collection. The volunteers were weighed and an intravenous cannula (Venflon 2, 18G) was placed into a vein in one forearm, for blood sampling. Volunteers then drank a further 200 ml of water and remained supine for 1 h. After 1 h 5 ml of blood was sampled for basal measurement of plasma renin activity and aldosterone. The volunteers then emptied their bladders again before receiving one of the following:

(1) 40 mg oral frusemide solution (10 mg/ml "Lasix" injection) as a single oral dose given in 50 ml of orange squash (10 ml Kia-Ora orange squash + 40 ml of water). They also received 50 ml of orange squash every hour for the next 8 h.

(2) 5 mg oral frusemide solution every hour for 8 h (10mg/ml "Lasix" injection). Hourly doses were given in 50 ml of orange squash (10 ml Kia-Ora orange squash + 40 ml of water).

A low salt lunch was provided between 5 and 6 h after dosing and a light evening meal was provided over the last hour of the study.

Fluid Replacement

Initially it was decided that fluid loss would be replaced orally with water according to the volume of urine passed in the previous hour. Under these conditions 2 volunteers started the study. However, because the volunteers passed such large volumes of urine after frusemide they had problems consuming equivalent amounts of water. During repeated dosing of frusemide, one subject also complained of a bad headache. He experienced the same symptoms during single dose administration and could not drink any more water after 7 h. At this point he decided to withdraw from the study.

The fluid replacement scheme was revised and the remaining volunteers repeated the study receiving 100 ml of water every hour. Further problems were found. Following the repeated doses of frusemide the volunteer experienced slight dizziness towards the end of the study. 2 h after administration of the single dose the same volunteer nearly fainted when standing to pass urine and needed to lie down. His standing blood pressure was checked and was found to be low. The study was abandoned and the volunteer was given 1000 ml of water to drink and 1 litre of 0.9 % saline was administered intravenously. Larger volumes of water were therefore needed to compensate for fluid loss.

The final fluid replacement scheme used for the 8 volunteers who participated in the study was 150 ml of water every half hour. This resulted in fewer side effects (See Results Section).

Blood Samples

a) Frusemide analysis

Venous blood samples (5 ml) were taken just before dosing and at 15, 30, 45, 60, 75, 90, 105, 120, 180, 240, 300, 360, 480 and 600 minutes after administration of the single dose. During repeated dosing blood sample (5 ml) were taken just before the first dose and then every 30 minutes up to 10 h. Samples were collected in lithium heparin tubes and centrifuged at 3000 rpm for 15 min. Plasma was stored at -20^{0}.

b) Plasma renin activity and aldosterone

On both occasions venous blood samples (5 ml) were taken every half-hour for the first 4 h and then hourly every hour up to 10 h. They were collected into tubes containing 200 µl of 5 % ethylenediaminetetra-acetic acid disodium salt (EDTA), which were kept on ice. After centrifugation at 4^{0} (2500 rpm for 20 min), the plasma was separated immediately and stored at -40^{0} until analysis.

Urine Collection

On both occasions urine was collected hourly until 10 h. After 10 h the volunteers went home, with no restriction on fluid intake, and collected urine from 10 to 24 h. Urine volumes were recorded and aliquots stored at -20^{0} until analysis.

Analysis of samples

Frusemide concentrations in plasma and urine were determined by high performance

liquid chromatography with fluorescence detection (Chapter 2). Plasma renin activity and aldosterone concentrations were measured by radioimmunoassay (Chapter 2). Urinary sodium and potassium were analysed by ion specific electrodes.

Analysis of Data

The $AUC_{0\text{-}10h}$ values for single and repeated doses of frusemide were calculated using the trapezoidal rule (See Chapter 2). Following the single oral dose, frusemide concentrations in plasma could in general only be measured up to 5 h after dosing. Plasma concentrations at 6, 8 and 10 h were estimated by linear regression.

Statistics

Data are expressed as means ± standard deviations (s.d.). Statistical comparisons were assessed by the use of the Student t test for paired data with a P value of <0.05 accepted as significant.

Section 9.3. Results

24 h sodium excretion values on the day prior to each study period were similar: 122.4 ± 30.0 mmol prior to the single dose of frusemide and 132.6 ± 37.2 prior to the repeated doses. The corresponding potassium excretion rates were 53.4 ±22.1 and 53.8 ± 18.7 mmol and these differences were not significant.

Mean frusemide plasma concentration time curves and mean urinary frusemide excretion rates following single and repeated doses are shown in Fig. 9.2. The $AUC_{0\text{-}10h}$ averaged 2.89 ± 0.66 mg.h/l when frusemide was administered as a single dose

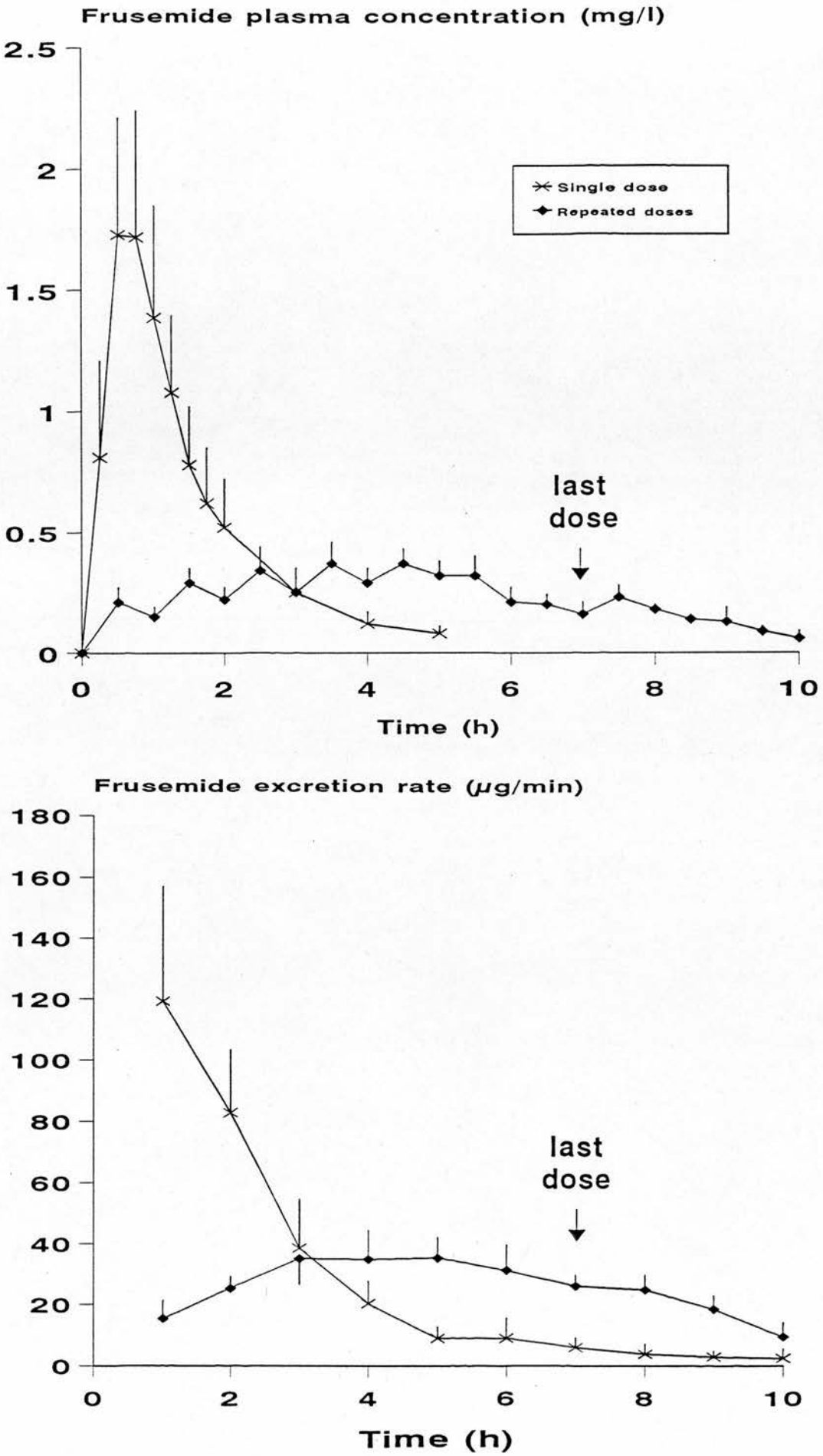

Figure 9.2 Mean frusemide plasma concentrations (top graph) and mean urinary frusemide excretion rate (bottom graph) after 40mg frusemide and 5 mg frusemide every hour for 8h. Bars = ± s.d.

compared with 2.25 ± 0.32 mg.h/l when administered as repeated doses (Table 9.1.) The difference was significant, $P<0.05$.

Urinary frusemide excretion rates following repeated administration increased up to 3 h, remained fairly constant for the next 2-3 h and progressively declined thereafter. By contrast, after the single dose a peak value of frusemide urinary excretion rate was observed during the first hour, followed by a decrease during subsequent hours. The total urinary recovery of frusemide over 24 h (Table 9.1.) following single and repeated doses were 18.3 ± 3.3 and 16.4 ± 3.0 mg, respectively ($P<0.05$). This corresponds to 45.8 ± 8.3 and 41.0 ± 7.6 % of the total dose administered.

Frusemide Response

Cumulative urinary sodium excretion and mean sodium excretion rates following single and repeated doses of frusemide are given in Figs. 9.3 and 9.4. Following the repeated doses of frusemide, there was a tendency towards higher total sodium output over 10 h compared to the single dose (Table 9.2). However the difference was not statistically significant.

After the single dose of frusemide, urinary excretion of sodium (Fig 9.4) decreased to below the baseline level by 5 h, indicating a period of net sodium retention. During the repeated doses sodium excretion rate did not reach a peak until the third hour. A gradual decline in natriuretic response then occurred, falling below baseline by 10 h.

Cumulative urinary volume and cumulative urinary potassium excretion are shown in Figs. 9.5 and 9.6. No significant differences were found in total potassium excretion and

Table 9.1. Area under the plasma concentration time curves and total urinary recovery of frusemide over 24 h (Ae) following single and repeated doses in 8 healthy volunteers.

Subject	$AUC_{0\text{-}10h}$ (mg.h/l)		Ae (mg)	
	Single	Repeated	Single	Repeated
1	3.19	2.52	20.02	19.17
2	2.03	2.49	18.36	18.77
3	3.40	2.55	23.12	19.57
4	2.26	1.82	20.94	17.86
5	3.31	1.87	17.31	16.05
6	2.65	2.14	16.66	12.35
7	2.35	2.04	11.16	10.74
8	3.89	2.56	18.95	16.93
Mean	2.89	2.25	18.32	16.43
±s.d.	0.66	0.32	3.32	3.05
P	<0.05		<0.05	

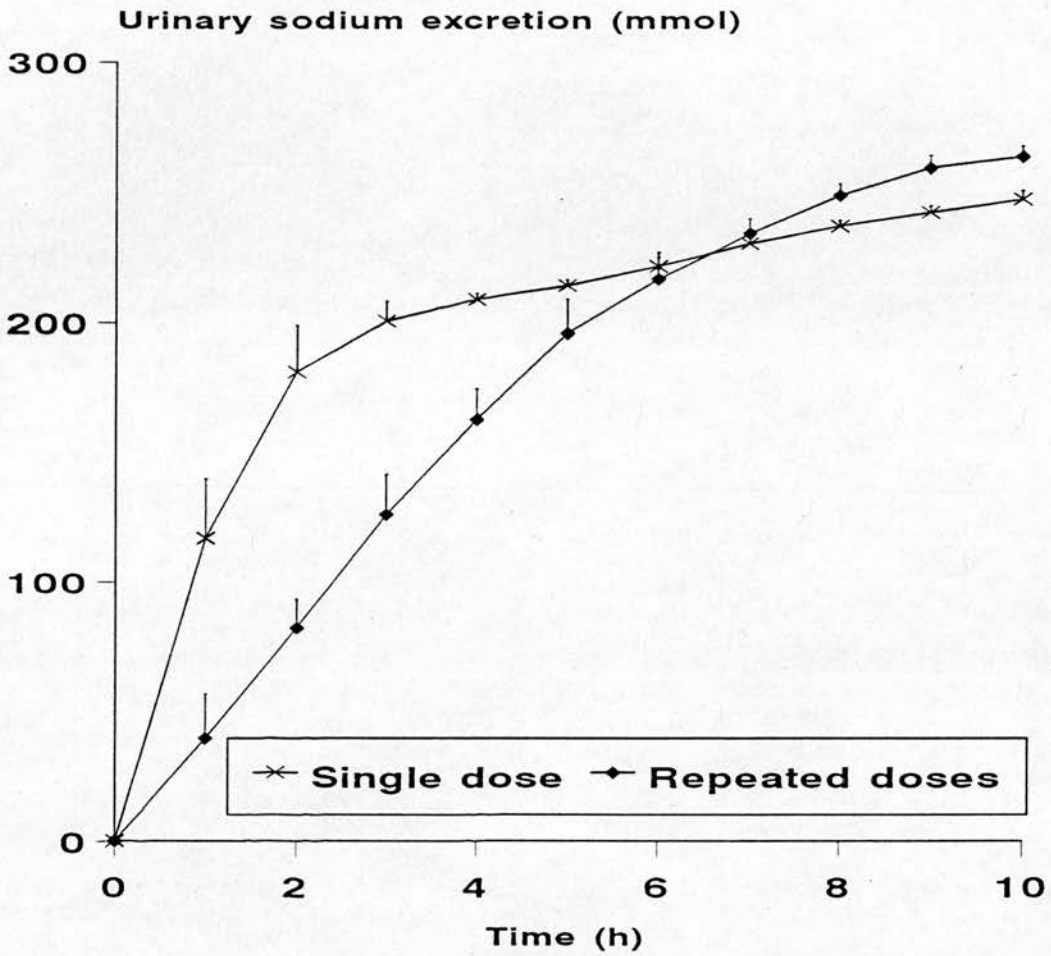

Figure 9.3. Cumulative urinary sodium excretion during 10h after single and repeated doses of frusemide. Bars = ± s.d.

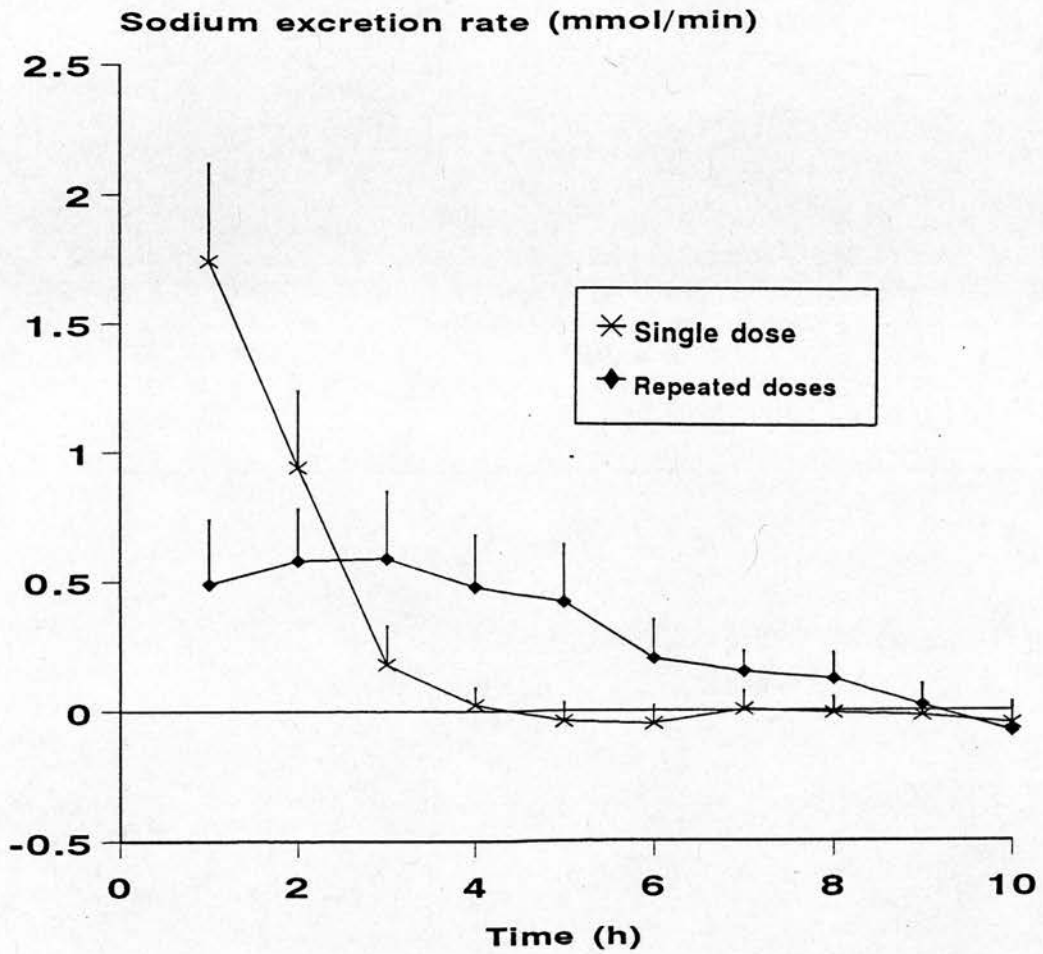

Figure 9.4 Net urinary sodium excretion rate versus time. Response is represented by urinary excretion rate over baseline. Values less than zero indicate sodium retention.

Table 9.2. Response to single and repeated doses of frusemide over 10 h in 8 healthy volunteers. U_{Na} is urinary sodium excretion, U_K is urinary potassium excretion.

Subject	U_{Na} (0-10h) (mmol)		U_K (0-10h) (mmol)		Urine volume (0-10h) (ml)	
	Single	Repeated	Single	Repeated	Single	Repeated
1	295.1	304.5	57.9	61.7	3540	3475
2	266.2	295.1	37.3	50.8	3069	3498
3	227.8	273.1	56.9	66.3	3814	4224
4	277.2	386.0	29.8	61.4	4135	5487
5	282.6	282.7	69.7	87.4	4778	4375
6	200.6	130.2	42.3	36.6	3830	3516
7	203.4	187.1	63.7	53.5	3204	3520
8	199.9	237.4	39.3	54.2	3867	4332
Mean	244.1	262.0	49.6	59.0	3780	4053
± s.d.	37.8	72.8	13.4	13.7	503	659
P	NS		NS		NS	

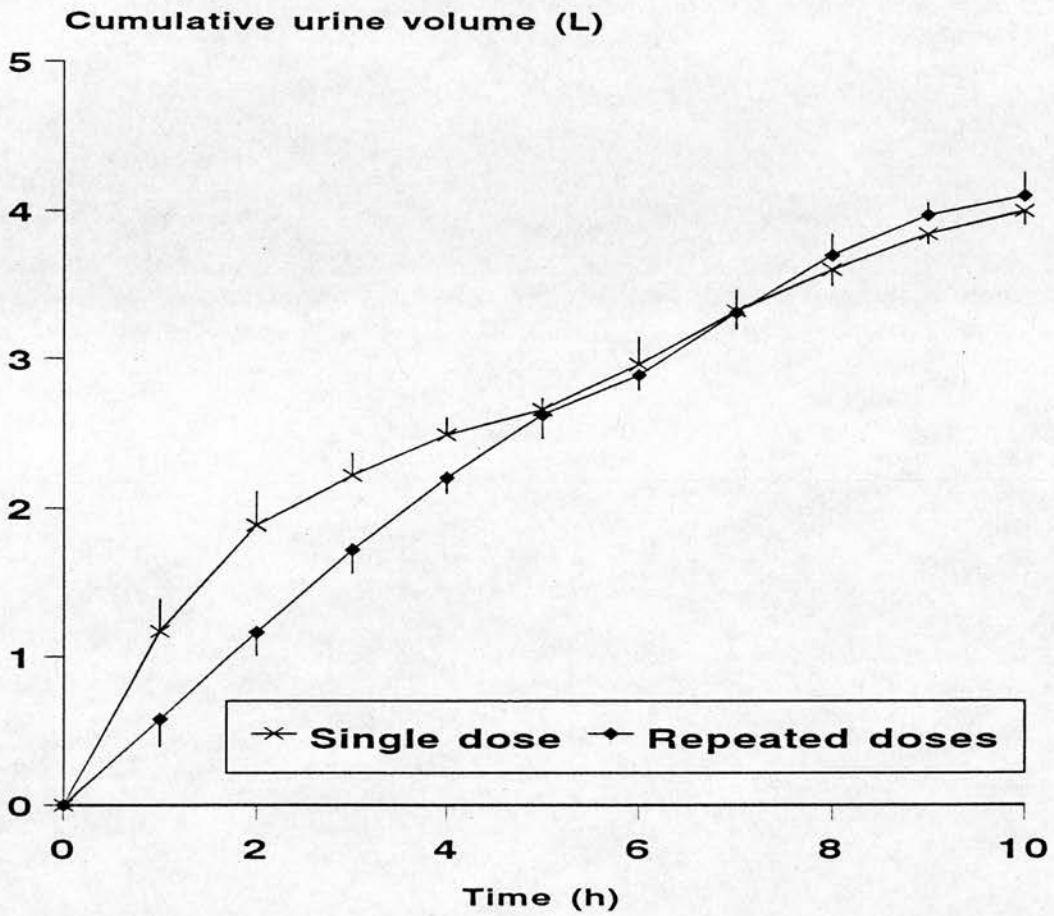

Figure 9.5. Cumulative urinary volume over 10h following administration of single and repeated doses of frusemide.

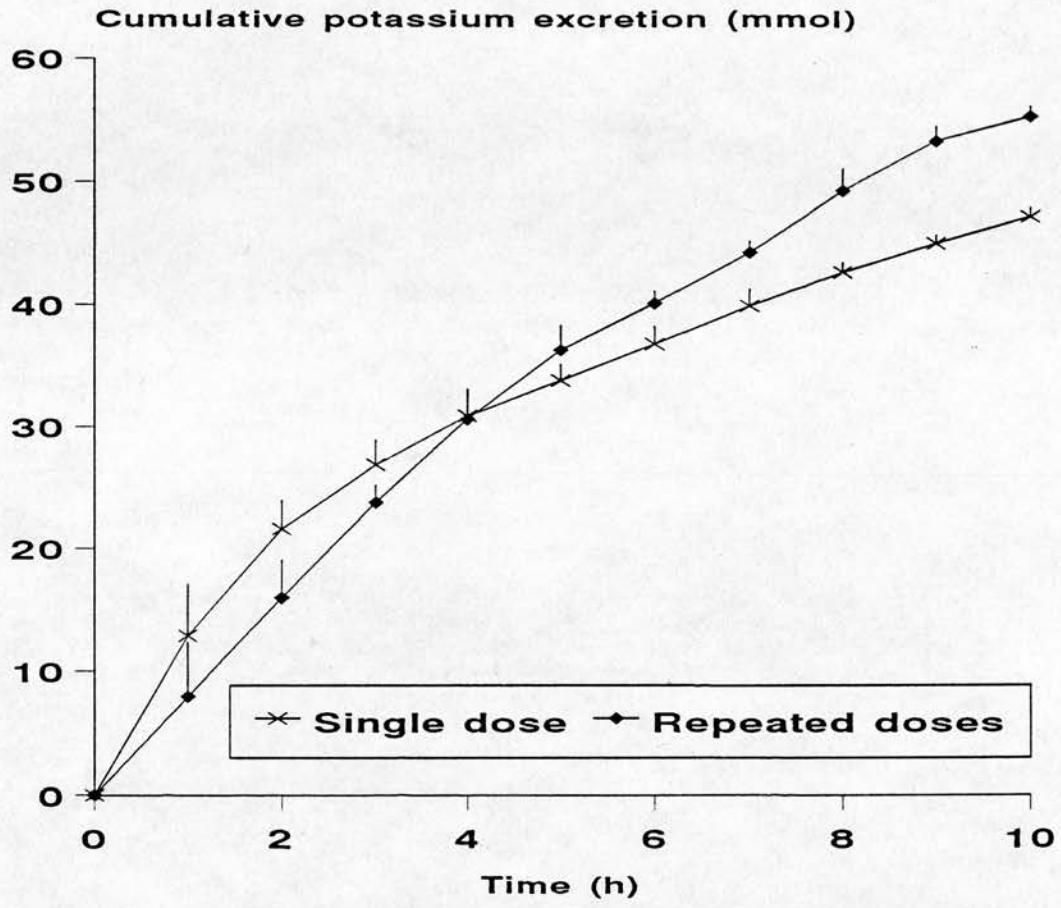

Figure 9.6. Cumulative potassium excretion over 10h following administration of single and repeated doses of frusemide.

urine output over 10 h following repeated and single doses of frusemide (Table 9.2)

Plasma renin activity (PRA) and Aldosterone

PRA and aldosterone concentrations following single and repeated doses of frusemide are shown in Figs. 9.7 and 9.8.

PRA

Basal measurements of PRA were similar on both study days: 1.94 ± 1.00 prior to the single dose and 1.91 ± 1.22 prior to the repeated doses. Following frusemide administration large interindividual variation was found but different patterns in PRA were evident. An early rise in PRA was found following the single dose of frusemide. Concentrations peaked at 1 h and gradually declined over the next 3 h but did not reach basal level PRA. A later smaller rise occurred at 6 h. By contrast, during the repeated doses of frusemide , a more gradual increase in PRA was found. From 5-10 h PRA was higher than after the single dose.

Aldosterone Concentrations

Plasma aldosterone concentrations followed a similar pattern to PRA during the single dose of frusemide. With the repeated doses plasma aldosterone fluctuated around the basal value showing an increase at 6 h.

Side Effects

Side effects consisted of dizziness and headache. Subjects 2, 3 and 6 experienced slight

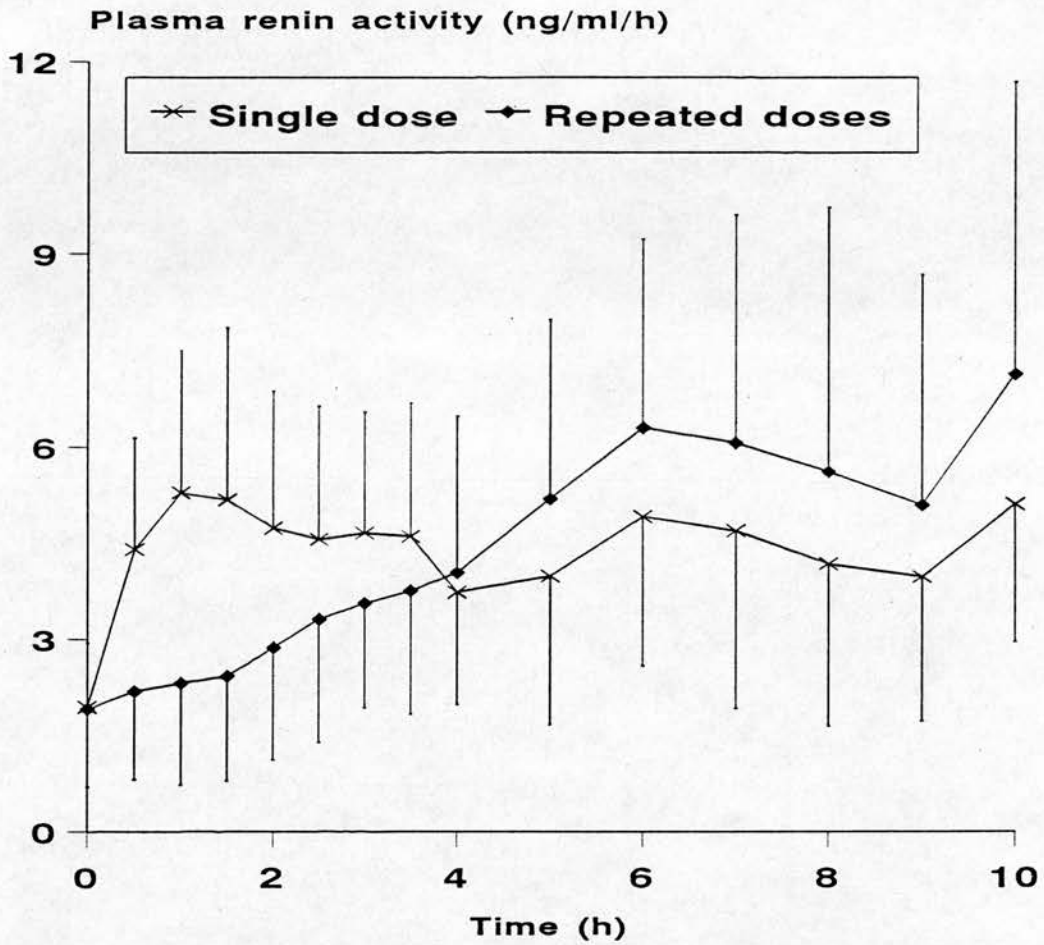

Figure 9.7. Plasma renin activity during administration of single and repeated doses of frusemide.

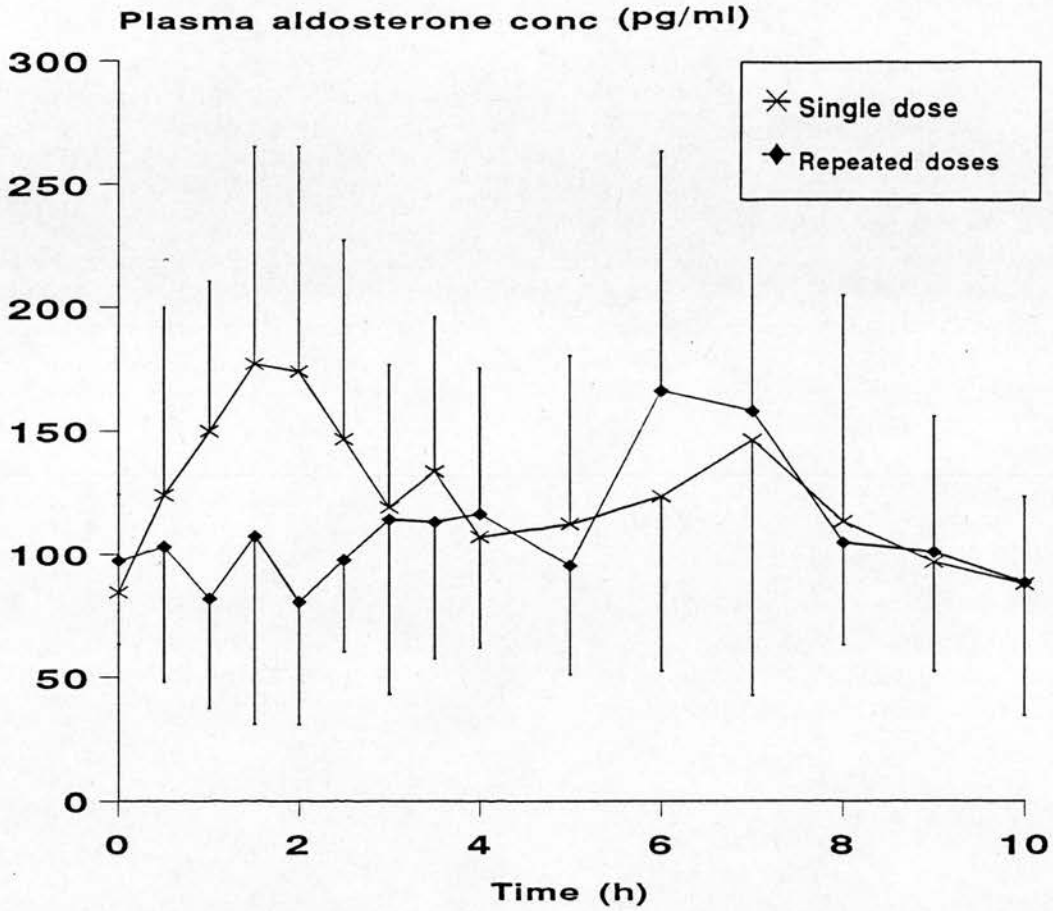

Figure 9.8 Plasma aldosterone concentrations during administration of single and repeated doses of frusemide.

dizziness at the end of the study day following the single dose of frusemide. Subject 3 also felt dizzy 3-4 hours after the start of the repeated doses and complained of a headache after 8 h.

Section 9.4. Discussion

The importance of fluid and/or electrolyte replacement for urine loss in the evaluation of diuretics has long been recognised (Branch *et al.* 1977; Hammarlund *et al.* 1985) and various types of rehydration schemes have been used. Kaojarern *et al* (1982) and Chennavasin *et al* (1980) employed intravenous isovolumetric replacement of all urine losses with Ringer's solution. Brater *et al* (1983b) and Noormohamed *et al* (1991) replaced urine volumes with equivalent amounts of water given orally. Li Kam Wa *et al* (1991) administered 100 ml of water orally every hour; Rudy *et al* (1991) and Alván *et al* (1988) administered 150 ml of water orally every hour; Hammarlund *et al* (1985) gave 200 ml of water per hour. In the present study problems were encountered with two fluid replacement schemes previously used by several other investigators (i.e. isovolumetric replacement of urine losses with water and 100 ml of water administered every hour). Using isovolumetric replacement of urine with water the volunteers were unable to drink the large quantities required, whereas 100 ml of water every hour appeared inadequate with one volunteer nearly fainting through a drop in blood pressure. Problems such as these were not reported by other investigators.

Most drugs are still given by injection and as conventional tablets and capsules. Due to its relatively short half-life, frusemide in these forms is short acting and potent which in some patients may lead to excessive diuresis, side effects and inconvenience.

Frusemide delivery rate in the nephron has been reported to be one of the major determinants of diuretic response (Kaojarern *et al.* 1982). According to this concept a low but continuously effective urinary excretion rate of frusemide may lead to a higher diuretic effect than short exposure of the active site to high concentrations of the drug. Several studies have already investigated whether continuous infusion of loop diuretics may be a more efficient way to administer this kind of drug.

In animals Lee *et al* (1986) compared different lengths of infusion time using the same total dose of frusemide. They found increased diuretic effect with increasing infusion times, whereas pharmacokinetic parameters were not significantly different between the four infusion times (10 s, 30 min, 1 h and 8 h). Increased diuretic effects with increasing infusion times were also reported when the same total dose of the loop diuretic bumetanide was infused for 10 s, 1 h and 4 h. This occurred despite lower urinary excretion of unchanged bumetanide following the 1 and 4 h infusions (Ryoo *et al.* 1993).

In 1983, Copeland *et al* compared the diuretic effects of the same dose of frusemide given by bolus and by constant infusion in 18 postoperative patients. A gentle sustained diuresis was achieved by constant infusion, but no significant difference was found in total urine volume and total sodium excretion compared with bolus frusemide administration. However several subsequent studies demonstrated superior efficacy of continuous intravenous infusion of loop diuretics. In all these studies the continuous infusions were preceded by small loading doses but again the same total dose of frusemide was administered compared to the bolus dose. Meyel *et al* (1992), in healthy volunteers, found a greater response after treatment with continuous infusion compared

to bolus injection, whereas total urinary frusemide showed no difference. Lahav *et al* (1992) carried out a similar study in patients with congestive heart failure. The continuous infusion produced diuresis and natriuresis that were significantly and consistently higher than intravenous administration. Furthermore, the infusion induced sustained diuresis with relatively little variation in urine output. Finally Rudy *et al* (1991) demonstrated that continuous infusion of the loop diuretic bumetanide also resulted in significantly greater net sodium excretion compared with bolus injection in eight patients with chronic renal failure. The greater overall natriuresis with continuous infusion compared to intravenous bolus injections may be attributed to the changed time course of delivery of frusemide into the urine.

Based on these observations and the concept of Kaojarern *et al* (1982) we decided to compare the effects of the same total dose of frusemide administered as a single oral dose and as repeated doses over 8 h in order to determine the benefits of another alternative and perhaps more efficient mode of therapy, slow or controlled release tablets.

The average $AUC_{0\text{-}10h}$ and total urinary recovery of frusemide over 24 h were found to be significantly lower following the repeated doses. Despite less drug being delivered to the active site no significant differences were found in total sodium, potassium and water excretion compared to the single oral dose. This cannot be attributable to dissimilar prestudy conditions since baseline sodium excretions were not significantly different before each phase of the study. The low but more prolonged urinary excretion rate of frusemide found during repeated administration may have produced more efficient drug utilisation than the single dose. In other words, more sodium output

relative to the excreted amount of diuretic drug.

After the single dose of frusemide sodium excretion rates fell below baseline at hour 5. During repeated dosing a gradual decline in natriuretic response occurred after 3 h despite the fairly constant urinary excretion of frusemide from 3-6 h. These data indicate that compensatory mechanisms are invoked even after excretion of relatively small amounts of sodium. However sodium excretion rates remained above baseline up to 9 h (2 h after the last dose) presumably because the diuretic was still being eliminated. The renin-angiotensin-aldosterone system may be responsible, at least in part, for this apparent rebound effect. Different trends in PRA and aldosterone were observed following single and repeated frusemide administration which were consistent with frusemide response. An early rise in PRA and aldosterone were found during the brisk natriuresis following single dose administration whereas a gradual rise in PRA was found following repeated administration which corresponds to the lower sodium excretion rates and gradual decline in sodium excretion found during this dosage regimen.

In conclusion, these results suggest that in terms of total diuretic and natriuretic response an oral sustained release formulation would offer no advantage over conventional tablets. No increased response was found using repeated dosing. Repeated dosing did however avoid the peak diuresis associated with a single dose without any loss in overall diuretic effect which may be of benefit to some patients using frusemide long term.

CHAPTER 10

FINAL DISCUSSION AND SUMMARY

Final Discussion

An important prerequisite for all bioavailability/pharmacokinetic studies is an accurate and reliable analytical method to measure drug concentrations in biological fluids. In the case of frusemide, the controversy surrounding its stability in acidic conditions and in light has led to the continued development of various assays employing several different extraction and detection techniques. Most have utilised liquid-liquid extraction for sample clean up and high performance liquid chromatography with ultraviolet or fluorescence detection which has provided good sensitivity and precision and fairly consistent results. However the use of strong acids (e.g. hydrochloric acid) in some liquid extractions together with long handling times and interfering endogenous peaks on some chromatograms despite laborious extraction steps, suggest that it may not be the most suitable procedure for the extraction of frusemide. To measure frusemide in plasma a relatively new solid phase extraction technique was therefore chosen which was developed by Russel *et al* in 1989 to try to circumvent some of these problems. The procedure is based on the adsorption of frusemide on the surface of a reversed-phase silica (C_{18} Bond Elut) column. Although the process was still fairly time consuming it did consistently yield final samples with few endogenous peaks, none of which interfered with frusemide or the internal standard, desmethylnaproxen. The process also proved to be highly selective for frusemide since no additional peaks were found in our patient samples despite the fact that they were all taking several other drugs. Frusemide recoveries of over 90 % also indicated that there were no major problems with acid hydrolysis or photochemical degradation during the extraction. Care was taken to exclude light by using amber glassware. In terms of sensitivity and accuracy, the method

did not differ from other published liquid-liquid extractions methods. However good linearity over the range 0.05 - 1 and 1 - 10 mg/l and acceptable precision at or above 0.05 mg/l (coefficient of variation less than 10 %) allowed for adequate quantification of plasma drug concentrations following administration of both therapeutic doses of frusemide (40 and 80 mg) and the low dose frusemide administered in the dose dependent and single versus repeated dose studies.

A similar extraction procedure was deemed unnecessary to measure frusemide in urine since good reproducibility and linearity were obtained by direct injection of urine onto the high performance liquid chromatography column. The day to day coefficient of variation varied between 4.3 and 1.4 % over the concentration range of 0.1 to 5.0 mg/l. The statistical agreement of frusemide bioavailability calculated from both plasma and urinary data in Chapter 3 suggest that plasma and urine determinations were both reliable and consistent. The stability tests indicated that frusemide was stable in plasma and urine frozen at -20^{o}C for at least 8 and 10 weeks, respectively. All samples were analysed well within this time period.

The assay of bumetanide is more difficult due to the fact that plasma concentrations after therapeutic doses are usually in the low ng/ml range. For plasma samples a method developed by Wells *et al* in 1991 was used, which again comprised solid phase extraction and fluorescence detection. Previous methods required large sample volumes, cumbersome extractions and dual detection of bumetanide and the internal standard. Compared to other assays it therefore provided a relatively simple approach to achieve separation of bumetanide without any loss of sensitivity and precision. It was possible to

measure concentrations as low as 2.5 ng/ml using 0.2 ml plasma samples, which was in fact lower than the 5 ng/ml limit quoted by Wells *et al* (1991). The day to day precision, as determined by coefficients of variation, did not exceed 8 % over the concentration range studied. As with frusemide, direct injection of urine samples provided satisfactory precision (coefficient of variation < 5 %) and good linearity over the range 0.1 - 1 mg/l. Bumetanide was stable in plasma and urine frozen at -20°C for up to 12 weeks and all plasma and urine samples were analysed using the above method within one week following collection.

Having set up reliable analytical methods to measure frusemide and bumetanide in plasma and urine, one of the first objectives was to compare the common experimental situation of giving these drugs in the fasting state with a clinically more relevant situation i.e. when taken together with food. Previous similar studies with frusemide had provided variable results (Beermann and Midskov, 1986; Hammarlund *et al.* 1984; Kelly *et al.* 1973) and no such studies had been carried out with bumetanide. Concomitant intake of breakfast delayed the absorption and significantly reduced the bioavailability of a 40 mg solution of frusemide given to eight healthy volunteers. Analogous results were reported by Beermann and Midskov (1986) who found that a similar breakfast reduced the bioavailability of a 40 mg frusemide tablet by approximately 30 % in healthy volunteers. The effect therefore appears to be independent of formulation. This contrasts with other drugs such as nitrofurantoin (Rosenberg and Bates, 1976), aspirin (Spiers and Malone, 1967; Volans, 1974) and erythromycin (Hirsch and Finland, 1959; Hirsch *et al.* 1960) where formulation has been shown to have a pronounced effect on the extent of the drug-food interaction,

suspensions and effervescent forms of these drugs were much less susceptible to the action of food that conventional tablets.

In contrast to the findings with frusemide, the absorption of bumetanide solution in healthy volunteers was delayed but not significantly reduced by the same breakfast. The reason why food appears to interact differently with bumetanide and frusemide remains unclear but may reflect differences in sites of absorption or metabolism. Other related drugs whose absorption has been found to be affected differently by food intake include bendroflumethiazide (Beermann *et al.* 1978) and hydrochlorothiazide (Beermann and Groschinsky-Grind, 1978) and ampicillin (Welling, 1977b) and amoxycillin (Melander, 1978). The bioavailabilities of hydrochlorothiazide and ampicillin are increased and reduced, respectively by food whereas it appears to have no influence on the absorption of either bendroflumethiazide or amoxycillin. Irrespective of the mechanisms involved, the lack of effect of food on bumetanide absorption may represent a therapeutic advantage of this drug which also had a somewhat greater bioavailability than frusemide (approximately 84 % versus 75 %). Although the bioavailability of oral bumetanide compared well with values reported by other investigators (Brater *et al.* 1983a, 1984; Cook *et al.* 1988; Holazo *et al.* 1984; Marcantonio *et al.* 1982), frusemide bioavailability was generally higher than most previously reported values. Although highly variable, its bioavailability usually falls in the range 40 - 60 % (Beermann *et al.* 1975; Brater *et al.* 1982; Hammarlund *et al.* 1984; Kelly *et al.* 1973; Ponto and Schoenwald, 1990). The higher bioavailability obtained in the present study may be due to administration of frusemide as an oral solution. Clinical evidence does support the therapeutic benefits of a liquid form of frusemide (content of vials for injection) given orally in patients with

heart failure (Niazov *et al.* 1985) although previous studies in healthy volunteers have suggested that tablet and solution forms of frusemide are equivalent in the extent of absorption (Waller *et al.* 1982, 1988; Hammarlund *et al.* 1984). In the above study oral administration of bumetanide, irrespective of the presence or absence of food and despite less drug reaching the urine, was also shown to elicit a greater pharmacological response than intravenous bumetanide. This effect may be due to the maintenance of an effective plasma concentration of bumetanide for a longer period after oral than intravenous administration.

One problem which arose in both of these studies was the inability to fit the plasma concentration time curves following frusemide administration with food to the pharmacokinetic model by computer due to the irregular absorption profiles. Instead, the $AUC_{0\text{-}8h}$ values were calculated using the trapezoidal rule. However the area beyond the last measured concentration to infinity had to be estimated using the intravenous elimination rate constant, assuming that the disposition kinetics of the drug would be similar after oral administration, and that there would be no change beyond this point. Because the potential error in doing this was small, this approach was felt to be justified.

The majority of patients subsequently surveyed in the medical wards of the Edinburgh Royal Infirmary were found to be taking a single early morning oral dose of frusemide with or in close proximity to breakfast, a situation where efficacy could clearly be compromised. However in spite of the findings in healthy volunteers, a standard hospital breakfast appeared to have no significant adverse effect on the absorption of frusemide

or its diuretic and natriuretic response in these patients. The lack of a significant effect may reflect the rather small number of patients studied since the areas under the plasma concentration time curve and urinary recoveries of frusemide were increased in six of the ten patients when the dose was administered 2 h after breakfast. Day to day variation in absorption and response may also have masked any significant improvement. Absorption of frusemide, especially in heart failure patients, is known to be even more erratic (Brater *et al.* 1982a, Greither *et al.* 1979). It is also possible that only certain types of food affect the uptake of frusemide. This has previously been shown for other drugs including paracetamol, the absorption of which is delayed to the greatest extent by a high fat breakfast (Jaffe *et al.* 1971; MacGilveray and Mattok, 1972; Walter-Sack *et al.* 1989; Wessels *et al.* 1992). Griseofulvin absorption is only increased by high fat meals, most probably due to food induced enhancement of dissolution (Welling, 1977b), and tetracycline absorption is reduced by calcium rich foods due to chelation with metal ions (Neuvonen, 1976; Neuvonen and Turakka, 1974). The absorption of frusemide should perhaps therefore have been tested in the presence of a high fat, a high carbohydrate and a high protein meal to establish the degree of interaction with each. Further investigations in larger patient populations are needed in order to fully determine the effect of food on frusemide absorption and more importantly the diuretic response under the usual clinical conditions of use. Overall however, these food-drug interaction studies suggest that patients should perhaps take frusemide on an empty stomach or at least in a standardised way in relation to meals in order to minimise variation in absorption and response. On the other hand, because bumetanide absorption was much less affected by food, there may be no need to control the time of its administration in

relation to meals.

No dose-dependent effect on frusemide absorption could be demonstrated over a range of doses commonly used in clinical practice as there were no significant differences in normalised plasma concentrations or areas under the plasma concentration time curves following administration of 10, 40 and 80 mg frusemide. Although it is impossible to predict what might happen outwith this dose range, the results obtained tend to dispute the evidence acquired in animals for site specific active absorption of frusemide (Chungi *et al.* 1979; Ritschel *et al.* 1991) and hence the hypothesis that site specific absorption may be one cause of the reduced bioavailability of frusemide (Ponto and Schoenwald, 1990). Other mechanisms, including poor solubility and presystemic metabolism, may be involved (Lee and Chiou, 1983; Ponto and Schoenwald, 1990). The failure of dose to influence frusemide clearance indicates that the disposition kinetics of frusemide were linear over the range 10 - 80 mg.

Early in vitro and in vivo animal studies demonstrated that frusemide exerts its primary diuretic action from the luminal surface of the ascending limb of the loop of Henle by inhibiting active chloride transport and thus preventing the reabsorption of sodium which passively follows chloride. This in turn results in a pronounced diuresis (Burg, 1976; Odlind, 1979; Odlind and Beermann, 1980; Rose *et al.* 1976; Seely and Dirks, 1977). Subsequent drug interaction studies with probenecid in healthy volunteers furthered the understanding of its mechanism of action. Pretreatment of normal subjects with probenecid caused a significant reduction in the renal and nonrenal clearance of frusemide and significantly increased plasma concentrations (Chennavasin *et al.* 1977;

Homeida *et al.* 1977; Honari *et al.* 1977; Odlind and Beermann, 1979). However while causing a rightward shift in the relationship between serum concentrations of frusemide and response, probenecid had no effect on the relationship between urinary frusemide excretion rate and response (Chennavasin *et al.* 1979). The conclusions from such studies was that the renal excretion of frusemide could be blocked effectively by probenecid acting to compete for the nonspecific organic acid active transport system situated in the proximal tubule. Also, the amount of frusemide delivered into the urine was more directly related to response than serum concentrations of frusemide. Therefore, agents which interfered with the renal tubular transport of frusemide should antagonise its diuretic action.

It was shown here that intravenous benzylpenicillin (Penicillin G), a drug commonly administered in large intravenous doses and which is eliminated from the body by active proximal tubular secretion (Kampmann *et al.* 1972; Martindale, 1993; Plaut *et al.* 1969), temporarily but significantly reduced the renal clearance and urinary excretion rate of frusemide, presumably by competing with it for renal secretion. The short lived effect was probably due to more rapid elimination of penicillin than frusemide, but this could not be confirmed as the rate of removal of penicillin was not determined. Interestingly, this pharmacokinetic interaction had no influence on either the rate or magnitude of the natriuretic and diuretic response to frusemide. This lack of effect on the response to frusemide was also found in some of the probenecid studies despite a much more constant degree of inhibition (Homeida *et al.* 1977; Honari *et al.* 1977), and in several instances the same total amount of frusemide in urine actually caused a greater overall natriuretic response following the administration of probenecid (Brater, 1978;

Chennavasin *et al.* 1979; Sommers *et al.* 1991). This unexpected finding was attributed to the changed time course of delivery of frusemide into urine (Brater, 1978, 1983; Kaojarern *et al.* 1982).

In agreement with the principle that the time course of delivery is important, Meyel *et al.* (1992) reported a significantly increased 8 and 24 h excretion of sodium when frusemide was given as a continuous infusion compared with an intravenous bolus injection of the same total dose in healthy volunteers. Similar results were obtained in another study by Lahav *et al* (1992) who demonstrated that continuous infusion of frusemide preceded by a bolus dose produced significantly greater diuresis and natriuresis than an equivalent total dose administered intermittently in patients with congestive heart failure. Based on these data it was thought that small repeated oral doses of frusemide (5 mg every h for 8h) might possibly/conceivably lead to a greater net sodium excretion than the equivalent single dose (40 mg). Although this hypothesis could not be substantiated in the present study in healthy volunteers, the repeated dosing regime did produce at least an equivalent diuretic and natriuretic response despite the delivery of significantly less drug to the urine over 24 h compared to the single dose. This pattern of prolonged delivery did seem therefore to produce a more efficient drug response. As discussed earlier, a similar discrepancy was observed in the bumetanide study where oral administration produced a greater pharmacological reponse than an equivalent intravenous dose in spite of a lower 24 h urinary recovery of bumetanide. Although this may be attributable to dissimilar prestudy conditions as no attempt was made to standardise sodium intake, similar observations have been noted by other investigators both in normal subjects (Holazo *et al.* 1984; Marcantonio *et al.* 1982) and in patients

with heart failure (Cook *et al.* 1988). These results seem therefore to provide further evidence that the diuretic and natriuretic reponse to frusemide and bumetanide is not directly related to the concentration of drug in urine and that a time factor may also be important.

Summary and Conclusions

1. Analytical methods developed by Russel *et al* (1989) and Wells *et al* (1991) were used to measure plasma concentrations of frusemide and bumetanide by solid phase extraction, HPLC separation and fluorescence detection, respectively. The sensitivity, reproducibility and linearity of these assays made them suitable for monitoring plasma and urine frusemide and bumetanide concentrations.

2. In 8 healthy volunteers given 40 mg oral frusemide, breakfast dramatically changed the pattern of the plasma concentration time curve compared to fasting administration. Mean peak concentrations were significantly reduced (2.35 ± 0.49 to 0.51 ±0.19 mg/l) and the mean time to peak concentration was delayed (0.69 ± 0.21 to 1.91 ± 0.93 h) indicating a reduced rate of absorption. Bioavailability, as judged from plasma and urine data was significantly reduced by approximately 30 %.

3. In a similar study with 2 mg oral bumetanide in 9 healthy volunteers, breakfast reduced the mean peak plasma concentration from 96.9 ± 15.1 fasting to 36.1 ± 11.5 µg/l, and it also delayed the mean time to peak from 0.53 ± 0.08 to 1.36 ± 0.72 h. Bioavailability however was not significantly reduced by food (74.8 ± 15.5 with and 83.7 ± 12.4 % without breakfast).

4. A survey of the timing of administration of frusemide in relation to breakfast was carried out over a 3 month period in the medical wards of the Edinburgh Royal Infirmary. Of patients who were prescribed a loop diuretic, 88 % were taking frusemide and 12 % bumetanide. Of patients taking oral frusemide, 76 % were given it as a single early

morning oral dose and the remainder took divided doses early in the morning and in the afternoon. The majority of patients (83 %) took the early morning dose during or within ± 10 minutes of the start or finish of breakfast. This survey demonstrated the extent to which the drugs are given in circumstances where efficacy may be compromised

5. In 10 patients receiving a single oral dose of at least 40 mg frusemide, significantly higher mean peak plasma concentrations were obtained following administration of their dose 2 h after breakfast (2.12 ± 0.95 mg/l) compared to administration at the usual time i.e. close to breakfast (1.47 ± 0.76 mg/l). However no significant differences were found in $AUC_{0\text{-}8h}$, total urinary recoveries or total natriuretic and diuretic response over 8 h when frusemide was given 2 h after breakfast.

6. Following administration of 10, 40 and 80 mg oral frusemide to 8 healthy volunteers on separate occasions, the normalised mean plasma concentrations (1.88 ± 0.63, 2.33 ± 0.54 and 2.13 ± 0.60 mg/l) and $AUC_{0\text{-}\infty}$ values were not significantly different (2.84 ± 1.13, 3.54 ± 0.88 and 3.40 ± 0.84 mg.h/l). Time to peak concentrations also did not differ significantly between the doses. Mean renal clearance values for the 10, 40 and 80 mg were 106.7 ± 39.8, 90.5 ± 32.0 and 99.0 ± 33.2 ml/min. These values were not significantly different. Hourly renal clearances for all doses were constant over 8 h. Frusemide does not exhibit dose-dependent absorption or clearance over the dose range 10 to 80 mg.

7. The mean renal clearance of frusemide was significantly reduced when administered in a dose of 40 mg together with 2.4 g intravenous benzylpenicillin in 8 healthy volunteers (103.0 ± 15.9 for frusemide alone versus 83.6 ± 17.4 ml/min for frusemide + penicillin).

This interaction could only be demonstrated during the first 2 h. Similarly, the urinary excretion rate of frusemide was correspondingly reduced over the first hour following administration with benzylpenicillin. The mean nonrenal clearance, plasma half-life, volume of distribution and $AUC_{0\text{-}6h}$ of frusemide were not altered by penicillin. Penicillin also had no effect on total sodium, potassium and water excretion

8. Another study was carried out in 8 healthy volunteers to compare the disposition and diuretic and natriuretic response of frusemide given as a single oral dose of 40 mg and as 5 mg every h for 8 h. The $AUC_{0\text{-}10h}$ (2.89 ± 0.66 to 2.25 ± 0.32 mg.h/l) and total urinary recovery of frusemide (18.3 ± 3.3 to 16.4 ± 3.0 mg) were significantly reduced following repeated dosing with 5 mg every h for 8 h compared to the single 40 mg dose. Despite these results there was a tendency towards higher urinary sodium following the repeated doses although the difference was not significant. A sustained release preparation of oral frusemide would thus offer no advantage over a conventional tablet. However taken together, these studies suggest that the time course of absorption and renal delivery of frusemide are important determinants of its diuretic and natriuretic effects.

Appendix 1

Patient Information

Patient Initials:

Date of Survey:

Date of Birth:

Date of Admission:

Sex:

Ward:

Patient Diagnosis:

Reason for Diuretic Treatment:

List of other drugs patient is receiving:

DRUG NAME	DOSE	ROUTE	TIMES GIVEN

Diuretic Information

Frusemide

Dose	Route	Time taken in relation to nearest meal:
20mg	Oral	_____
40mg		_____
80mg	Intravenous	_____
120mg		_____
Other		_____

Bumetanide

Dose	Route	Time taken in relation to nearest meal:
1mg	Oral	_____
2mg	Intravenous	_____
Other		_____

Has dose been changed since admission?: Yes / No

Increased / Decreased

If Yes, reason for change:

Was patient taking the same diuretic at home?:

Yes / No

If Yes, time taken in relation to nearest meal:

Appendix 2

Results from Diuretic Utilisation Survey

Patient No.	Age and Sex	Reason for diuretic	Diuretic	Dose	Times of administration	Dose changed since admission
1	84 F	Renal failure	Frusemide	40mg	5-10 min. after breakfast	50mg iv to 80mg oral to 40mg oral
2	84 F	Hypertension	Frusemide	40mg	With breakfast	No
3	66 F	Pulmonary oedema	Frusemide	80mg&40mg	With breakfast and 4pm	No
4	72 F	Oedema	Frusemide	40mg	2 min. after breakfast	No
5	89 F	Oedema	Frusemide	60mg	With breakfast	No
6	63 F	CCF	Frusemide	40mg	With breakfast	No
7	74 F	Pulmonary oedema	Frusemide	80mg	15 min. before breakfast	40mg oral to 80mg oral
8	87 F	Ankle oedema	Frusemide	40mg	Straight after breakfast	No
9	65 F	CCF	Frusemide	80mg bd	25 min. after breakfast &2pm	100mg iv bd to 80mg oral
10	68 F	CCF	Frusemide	40mg	Straight after breakfast	No
11	73 F	LVF	Frusemide	80mg	After one mouthful of tea	No
12	76 F	Renal failure	Frusemide	80mg	10 min. before breakfast	No
13	79 F	Pulmonary oedema	Frusemide	80mg	Half an hour after breakfast	No
14	80 F	Oedema	Frusemide	40mg	With breakfast	No
15	89 F	CCF	Frusemide	40mg	5 min. before breakfast	No
16	84 F	Pulmonary oedema	Frusemide	40mg	5-10 min. after breakfast	No
17	75 F	Peripheral oedema	Frusemide	40mg	25 min. before breakfast	No
18	74 F	Pulmonary oedema	Frusemide	40mg	10 min. before breakfast	80mg oral to 40mg oral
19	68 F	Hypertension	Frusemide	80mg	Straight after breakfast	No
20	74 F	Hypertension	Frusemide	40mg	10 min. after breakfast	No
21	80 F	Ankle oedema	Frusemide	40mg	With breakfast	No
22	82 F	Pulmonary oedema	Frusemide	80mg	10 min. after breakfast	40mg oral to 80mg oral
23	78 F	Pulmonary oedema	Frusemide	80mg	With breakfast	No

Patient No.	Age and Sex	Reason for diuretic	Diuretic	Dose	Times of administration	Dose changed since admission
24	71 F	Hypertension	Frusemide	40mg	10 min. before breakfast	No
25	62 F	Lymphoedema	Frusemide	80mg	Straight after breakfast	No
26	84 F	Hypertension	Frusemide	40mg	10 min. before breakfast	No
27	82 F	Ankle oedema	Frusemide	40mg	3 min. after breakfast	No
28	73 F	Hypertension	Bumetanide	1mg	Straight after breakfast	No
29	69 F	Pulmonary oedema	Frusemide	40mg bd	5 min. before breakfast &6pm	50mg iv on admission to 40mg oral
30	72 F	Hypertension	Frusemide	80mg&40mg	5 min. before breakfast &4pm	No
31	79 F	Peripheral oedema	Bumetanide	2mg	With breakfast	3mg oral to 2mg oral
32	87 F	Pulmonary oedema	Frusemide	120mg	5 min. before breakfast	80mg oral to 120mg oral
33	65 F	Hypertension	Frusemide	40mg	15 min. after breakfast	No
34	63 F	Pulmonary oedema	Frusemide	80mg&40mg	With breakfast &4pm	No
35	85 F	Pulmonary oedema	Frusemide	40mg	Straight after breakfast	No
36	68 F	Renal failure	Bumetanide	4mg bd	Just before breakfast &6pm	No
37	82 F	Pulmonary oedema	Frusemide*	80mg	15 min.after breakfast	No
38	75 F	LVF	Frusemide	40mg	With breakfast	No
39	56 F	Peripheral oedema	Frusemide*	80mg	With breakfast	120mg oral to 80mg iv
40	92 F	Pulmonary oedema	Frusemide	80mg&40mg	With breakfast &4pm	On admission 80mg iv infused over 1h
41	87 F	CCF	Bumetanide	3mg&2mg	With breakfast &4pm	No
42	68 F	Pulmonary oedema	Frusemide	40mg	5 min. before breakfast	50mg iv on admission to 40mg oral
43	65 F	Hypertension	Bumetanide	2mg bd	With breakfast and 6pm	No
44	86 M	CCF	Frusemide	120mg&80mg	15 min. before breakfast &4pm	No
45	82 M	Oedema	Frusemide	20mg	In spoonful of porridge	No
46	82 M	LVF	Frusemide	40mg	15 min. before breakfast	No
47	82 M	Cardiac failure	Frusemide	80mg	15 min. before breakfast	40mg oral to 80mg oral
48	76 M	Hypertension	Frusemide	40mg bd	15 min. before breakfast &6pm	No

Patient No.	Age and Sex	Reason for diuretic	Diuretic	Dose	Times of administration	Dose changed since admission
49	79 M	Cardiac failure	Bumetanide	1mg	With milk before porridge	No
50	76 M	Pulmonary oedema	Frusemide	80mg	Immediately after breakfast	40mg bd to 80mg morning dose
51	71 M	LVF	Bumetanide	2mg	5-10 min. after breakfast	No
52	82 M	CCF	Frusemide	80mg	5 min. after breakfast	40mg oral to 80mg oral
53	93 M	CCF	Frusemide	40mg	10 min. before breakfast	80mg oral to 40mg oral
54	76 M	Pulmonary oedema	Frusemide	40mg bd	10 min. before breakfast &2pm	No
55	67 M	Cardiac failure	Frusemide	80mg bd	5 min. before breakfast &4pm	No
56	81 M	Pulmonary oedema	Frusemide	60mg	20 min. after breakfast	No
57	63 M	Renal failure	Frusemide	500mg bd	2 min. after breakfast &10pm	No
58	54 M	Cardiac failure	Frusemide	80mg bd	Just after breakfast&2pm	No
59	86 M	LVF	Frusemide	40mg	Immediately after breakfast	No
60	84 M	Oedema	Frusemide	40mg	10 min. before breakfast	No
61	71 M	Pulmonary oedema	Frusemide	80mg bd	20 min. before breakfast&2pm	No
62	54 M	CCF	Frusemide	120mg	With breakfast	80mg oral to 120mg oral
63	66 M	Pulmonary oedema	Frusemide	80mg	Straight after breakfast	50mg iv on admission to 80mg oral
64	82 M	Pulmonary oedema	Frusemide*	80mg bd	Just before breakfast&2pm	50mg iv on admission to 80mg iv bd
65	53 M	Hypertension	Bumetanide	4mg bd	With breakfast & 6pm	No
66	58 M	Hypertension	Frusemide	80mg	5 min. before breakfast	No
67	72 M	LVF	Frusemide	40mg	With breakfast	No
68	76 M	Pulmonary oedema	Frusemide	80mg	10 min. before breakfast	50mg iv on admission to 80mg oral
69	73 M	LVF	Frusemide	80mg	5 min. before breakfast	No
70	72 M	LVF	Frusemide	40mg	With breakfast	50mg iv on admission to 40mg oral
71	77 M	LVF	Frusemide	40mg	5 min. before breakfast	No
72	83 M	Pulmonary oedema	Frusemide	80mg	With breakfast	50mg iv thrice on admission to 80mg oral
73	81 M	Ankle oedema	Frusemide	40mg	15 min. before breakfast	No

Patient No.	Age and Sex	Reason for diuretic	Diuretic	Dose	Times of administration	Dose changed since admission
74	71 M	Pulmonary oedema	Frusemide	80mg	Straight after breakfast	100mg iv on admission to 80mg oral
75	67 M	Hypertension	Bumetanide	2.5mg	Straight after breakfast	No

Note: iv=intravenous dose; CCF = congestive cardiac failure; LVF = left vetricular failure; * = intravenous dose

Patient No. Other prescribed drugs

1	Cimetidine 800mg om, Salbutamol 2 puffs pm, Temazepam 10mg on.
2	Aspirin EC 300mg om,Enalapril 5mg om ,Gaviscon 10ml as req.,GTN 1 spray as req.,Trimethoprim 200mg bd, Warfarin.
3	Diazepam 5mg om,Ferrous sulphate 200mg bd,Folic acid 5mg om,Methyl Dopa 125mg bd, Ranitidine 300mg bd.
4	No other drugs
5	Amitriptylene 75mg om,Bisacodyl 2 tabs bd,Codeine phosphate 30mg qid,Metoclopramide 10mg 8 hourly.
6	Aspirin 300mg om,Diazepam 5mg om,Diltiazem 90mg bd,GTN 2 sprays as req., ISMO 20mg bd,Metoprolol 25mg bd.
7	Enalapril 20mg 10pm,Ferrous sulphate 200mg bd,Glibenclamide 5mg om,Paracetamol 500mg td.
8	Cimetidine 400mg 10pm, Codydramol 2 every 6 hours.
9	Becotide bd,Digoxin 250mg 6pm,Enalapril 10mg 10pm.
10	Amiloride 5mg om,Amoxil 250mg tid,Cimetidine 800mg 10pm, Paracetamol 2 tabs as req.
11	Aspirin 75mg om,Digoxin 0,625mg om, Enalapril 10mg om,GTN 2 sprays as req., Nifedipine qid.
12	Diltiazem 60mg bd, GTN 2 sprays as req., ISMO 10mg bd, Temazepam 10mg 10pm.
13	Adalat LA 30mg om, Amiloride 5mg om ,Aspirin 75mg om,Digoxin 250mg om , Paracetamol 1g 6 hourly.
14	Aspirin 300mg om, GTN 2 sprays as req., ISMO 20mg bd, Nifedipine 10mg bd,Quinine sulphate 300mg om.
15	Coproxamol 2 tabs 6 hourly, Dihydrocodeine 30mg qid, Metoclopramide 10mg 8 hourly, Oxygen 35% continuous.
16	Amiloride 5mg om,Amoxycillin 500mg tid,Erythromycin 500mg tid,Paracetamol 2 tab as req,Prednisolone 10mg om,Salbutamol 2.5mg bd
17	Amiloride 5mg om, Becotide 100mg bd, Ipratropium 500mg qid,Paracetamol 1g 6 hourly, Salbutamol 5mg qid.
18	Augmentin tid,Coproxamol 2x6 hourly,Enalapril 10mg om,Erythromycin 250mg qid,GTN,Mucogel 20ml ,Ranitidine 150mg bd.
19	Aspirin 75mg om,Atenolol 100mg om,Enalapril 15mg bd,Glibenese 5mg om,Nitrazepam 5mg 10pm.
20	Amiloride 5mg om, Coproxamol 2 tabs 6-8 hourly, GTN 2 sprays,Thyroxine 150mg om.
21	Amiloride 5mg om,Aspirin 75mg om,Diltiazem 90mg bd, GTN 2 sprays pm, ISMO 20mg bd,Paracetamol 1g qid.
22	Amiloride 5mg om,Amoxil 500mg tid, Becotide 200µg bd,Digoxin 125mg 6pm, GTN,ISMO 10mg bd,Ventolin spray qid.
23	Aspirin 150mg om, Becotide 2 puffs, GTN 2 sprays., Ventolin 2 puffs.

Patient No. Other prescribed drugs

24	Amiloride 5mg om, Aspirin 75mg om, Augmentin 2 tabs tid, Coproxamol 2 tabs 4 hourly.
25	Slow K 1 tab tid.
26	Aspirin 75mg om, Digoxin 0.125mg om, Enalapril 20mg om.
27	Amoxil 500mg tid, Atrovent 500mg qid, Cocodamol 2 tabs qid, Erythromycin 500mg qid, Pulmacort bd, Senna 2 tabs 6pm.
28	Aspirin 75mg om, Atenolol 50mg om, Ranitidine 150mg bd, Slow K 1 tab bd.
29	Digoxin 125mg 2pm, Losec 20mg om, Temazepam 20mg 10pm.
30	Aspirin 300mg om,Diltiazem 60mg tid,Enalapril 15mg om,Imdur 60mg om,Metoclopramide 10mg 8 hourly,Temazepam 10mg 10pm.
31	Amiodarone 200mg om,Enalapril 20mg om, GTN 2 sprays as req.
32	Amiloride 5mg om,Aspirin 75mg om,Buscard Buccal 5mg om ,Coproxamol 2 x 6 hourly,Diltiazem 60mg tid,GTN,Salbutamol 2.5mg qid.
33	Digoxin 0.25mg om,Methyl Dopa tid,Paracetamol 1h 4-6 hourly, Temazepam 10mg 10pm.
34	Aspirin 75mg om, Cimetidine 400mg bd,Enalapril 10mg om,GTN 2 sprays pm, Quinine sulphate 30mg 10pm.
35	Amiloride 5mg om,Aspirin 300mg om,Diltiazem 60mg bd,GTN 2 sprays pm.
36	Adalat 20mg bd,Allopurinol 100mg om,Amiloride 5mg om,Codeine 30mg om,Methyl Dopa 250mg tid,Omeprazole 20mg om.
37	Amalodipine 5mg om,ISMO 40mg bd,Slow K 1 tab bd,Thioradizine 25mg 6pm.
38	Allopurinol 300mg om,Amlodipine 5mg om,Aspirin 300mg om,Digoxin 0.125mg om,Enalapril 10mg om,Gammanil,Phenobarbitone 60mg
39	Flucloxacillin 500mg qid,Penicillin V 500mg qid.
40	Aspirin 150mg om,ISMO 20mg bd,Lisinopril 10mg om.
41	Amoxycillin 250mg om,Captopril 25mg tid, Paracetamol 1g 6 hourly.
42	Augmentin 1.2g tid, Coproxamol 2 x 6 hourly, Enalapril 5mg om,GTN ,Imodium 2mg om, Nystatin lozenge tid,Vancomycin 1g bd.
43	Aspirin 150mg om,Enalapril 10mg bd,GTN 2 sprays as req., Paracetamol 0.5g 6 hourly.
44	Cimetidine 400mg om,Digoxin 62.5µg om,Gaviscon 10ml as req., Gliquidone 15mg om,ISMO 10mg bd.
45	Digoxin 0.125g om,Thioridazine 10mg bd, Whisky 30ml 6pm.
46	Amiloride 5mg om, Aspirin 75mg om,Paracetamol 1g 4-6 hourly, Senna 2 tabs 6pm.
47	Amiloride 5mg om,Diltiazem 60mg tid,Glipizide 10mg,GTN 2 sprays,ISMO 40mg tid.
48	Aspirin EC 300mg om,Diltiazem 90mg bd, Gaviscon 10ml as req., GTN 2 sprays ,ISMO bd,Slow K 2 tabs bd.

Patient No.	Other prescribed drugs
49	Aspirin 75mg om,Coproxamol 2 tabs tid,GTN 500µg as req., Metoprolol 50mg bd,Phenobarbitone 30mg tid.
50	Aspirin 75mg om,Enalapril 20mg om,Glipizide 10mg bd,GTN 2 sprays as req., ISMO 20mg bd,Lactulose 15ml bd.
51	Captopril 50mg om,Coproxamol 2 tabs 6 hourly, Sodium bicarbonate 1g tid, Temazepam 20mg 10pm.
52	Lisinopril 5mg om,Phenobarbitone 30mg tid,Phenytoin 50mg tid,Salbutamol 2 puffs as req.
53	Aspirin 300mg om,Enalapril 10mg om.
54	Amoxycillin 500mg tid,Aspirin 75mg om, Enalapril 20mg om,Glipizide 10mg bd,ISMO 20mg 6pm.
55	Aspirin 300mg om,Coproxamol as req,Hydralazine tid,Indomethacin 25mg tid,ISMO 20mg bd,Nifedipine 10mg om.
56	Amiodarone 200mg bd,Aspirin 300mg om,Augmentin1tid,Bisacodyl 5mg 10pm,Coproxamol 2 4-6 hourly,Omeprazole 20mg om.
57	Calcichew 500mg bd,Cephalexin 500mg bd,Gliquidone 30mg bd,Sodium bicarbonate 100mg 6pm, Ventolin 2mg bd.
58	Aspirin EC 300mg om,Codydramol 2x6 hourly,Enalapril 10mg om,Enalapril 20mg 6pm,Slow K 2 tab,Temazepam 10mg 10pm.
59	Captopril 12.5mg tid, Nifedipine 10mg bd.
60	Amiloride 5mg om, Amoxycillin 250mg tid,Aspirin 150mg om, Erythromycin 500mg qid.
61	Aspirin 75mg om,Captopril 25mg bd,GTN 2 sprays as req., ISMO 40mg bd,Paracetamol 1g.
62	Enalapril 5mg om,Temazepam 10mg 10pm, Warfarin as charted.
63	Adalat retard 20mg bd, Aspirin 300mg om, GTN as req., ISMO 20mg bd, Paracetamol 1g 6 hourly.
64	Diazepam 2mg 10pm,Digoxin 0.125mg 6pm,ISMO 40mg bd,Nifedipine 20mg bd,Paracetamol 1g 4-6 hourly.
65	Amiodarone 200mg om, Amoxycillin 500mg tid, Aspirin 300mg om, Captopril 50mg bd, ISMO 60mg om, Paracetamol 1g 4-6 hourly.
66	Aspirin 300mg om, Enalapril 10mg om, Oxygen as required .
67	Enalapril 10mg om Nystatin syrup 1ml tid.
68	Aspirin 75mg om,Digoxin 0.25mg om, Diltiazem 60mg om, GTN 2 sprays as req., ISMO 20mg bd, Paracetamol 1g 6 hourly.
69	Amitriptylene 25mg bd, Augmentin 1 tab bd, Chlordiazepoxide 10mg tid, Digoxin 250µg 2pm, Slow K 600mg bd.
70	Aspirin 300mg om, Digoxin 0.25mg 2pm, GTN 500µg as req., ISMO 20mg bd, Slow K 2 tabs bd.
71	Dihydrocodeine 30mg 6-8 hourly, ISMO 20mg bd, Senna 2 tabs pm, Slow K 2 tabs tid, Temazepam 10mg 10pm.
72	Aspirin 75mg om, Cyclimorph 5mg iv., Enalapril 5mg 10pm.
73	Aspirin 150mg om, Coproxamol 2 tabs 6 hourly.

Patient No. Other prescribed drugs

74 Amoxycillin 500mg bd, Aspirin 75mg om, Captopril 25mg om, Diltiazem 60mg tid, GTN infusion, ISMO 20mg bd.
75 Captopril 25mg tid, Gaviscon 10ml 4 hourly, Surgam 300mg bd.

Note: om = every morning; bd = twice daily; tid = three times daily; qid = four times daily

Appendix 3

A. **Foods to avoid:** (These contain a lot of salt)

Salted butter, salted margarine
Ordinary cheese
Bacon, ham, salami, sausages, black pudding
Tinned meat e.g. corned beef, spam, tongue, luncheon meat
Tinned fish e.g. sardines, tuna
Smoked fish e.g. kippers, golden cutlet (yellow haddock)
Meat and fish pastes
Tinned vegetables (some varieties e.g. Del Monte are now salt free and therefore suitable)
Tinned and packet soups
Commercially prepared sauces and ketchups
Oxo, Bovril, Marmite, Soya sauce, stock cubes
Salted crisps, salted nuts
Salted biscuits e.g. Tuc, Cheddars
Health salts e.g. Andrews

B. **Foods to be taken freely:**

Suitable meats: mince, stew, steak, chops, liver; roasting beef, lamb or pork; chicken, turkey; whiting, haddock, plaice, cod, sole, herring.
Fruit - fresh, stewed and tinned
Vegetables - raw or cooked using a little salt
Potato - cooked with a little salt
Nuts - unsalted
Breakfast cereal
Rice and pasta (boiled with a little salt)
Unsalted butter and low salt margarine
Oil, cooking fat
Jam, marmalade, honey, sugar
Boiled sweets
Tea, coffee
Eggs
Bread
Biscuits (avoid salty variety e.g. Tuc, Cheddar)
Ice cream, chocolate
Milk (not more than 1 pint per day)

Salt could be used in cooking but not sprinkled on the food afterwards (except on boiled egg or cold meat or salad where salt cannot be incorporated in the cooking - a little salt may be sprinkled on these items). Food taken with less salt than normal is improved by the use of herbs, spices, onion, garlic etc.

List of Abbreviations

Ae:	Total amount of drug excreted
AUC:	Area under the plasma concentration-time curve
C:	Drug concentration
Cmax	Peak plasma concentration
CL:	Clearance
CL_R:	Renal clearance
CL_{NR}:	Nonrenal clearance
C.V.:	Coefficient of variation
g:	Gram
h:	Hours
hplc:	High performance liquid chromatography
i.v.:	Intravenous
k_a:	Absorption rate constant
k_d:	Distribution rate constant
k_{el}:	Elimination rate constant
kg:	Kilogram
l or L:	Litre
µl:	Microlitre
mg:	Milligram
min.:	Minute
ml:	Millilitre
s:	Second
S.D.:	Standard deviation
Tmax:	Time to peak plasma concentration
$t_{½}$:	Elimination half-life
Vd:	Volume of distribution
yr:	year

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